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Impact of dietary fat composition on digestion, metabolism and deposition of fat in the growing pig

Trey Allen Kellner
Iowa State University

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Trey A. Kellner

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Program of Study Committee:
John F. Patience, Major Professor
Lance H. Baumgard
Brian J. Kerr
Kenneth J. Prusa
Kenneth J. Stalder

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DEDICATION

To my wife, thanks for your daily motivation and support, you truly are my balance in life.

To my mother and father, without giving me years of guidance and support, I would be unable to have the opportunity to publish these data. Words cannot justify how grateful and appreciative of my upbringing I am.

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ABSTRACT

The overall objective of this dissertation was to discern which components of the chemical composition of dietary fat affects the digestibility and energy content of dietary fat; the expression of genes involved in lipid metabolism; and the resulting carcass fat composition. Chapter 2, validated that dietary linoleic acid concentration was a more accurate predictor of carcass iodine value than iodine value product. Chapter 3, found that the addition of an unsaturated versus a saturated fat source did not alleviate the negative impacts associated with heat stress. Increased saturated fatty acid intake compared to increased intake of omega-6 fatty acids was found to decrease the mRNA abundance of fatty acid synthase (*FASN*) in adipose tissue in both Chapters 3 and 4. Decreased *FASN* mRNA content due to increased intake of saturated fatty acids would suggest that the de novo lipogenesis rate in adipose tissue is decreased. It was found in Chapter 5, that the DE content of dietary fat can be explained to a large degree by the chemical composition of dietary fat. However, the relationship between dietary fat DE content and its chemical composition was not the same between 13 and 50 kg of BW, respectively. In Chapter 6, it was found that the endogenous losses of fat accounted for 43% and 68% of fecal acid hydrolyzed ether extract at 13 and 50 kg of BW, respectively. Consequently, implying that the DE content of dietary fat is underestimated by 0.42 and 0.60 Mcal/kg, at 13 and 50 kg of BW, respectively, when measured on an apparent basis. This dissertation indicates that more work is needed to validate the DE, ME and NE estimates of dietary fat; to determine if dietary fat DE content needs to be adjusted for endogenous losses; to build a model that uses the chemical composition of dietary fat source and the energy intake of the pig, to explain observed lipid deposition rates and carcass fatty acid composition.

CHAPTER I

LITERATURE REVIEW: UTILIZATION OF DIETARY LIPIDS IN THE GROWING AND FINISHING PIG

Introduction

Dietary fats and oils are a highly digestible concentrated source of energy associated with a low heat increment (Forbes and Swift, 1944; Stahly et al., 1981). Dietary fat sources that can be included in swine diets are extremely diverse in chemical structure (Jorgensen et al., 2000). The majority of dietary lipids are consumed either as triacylglycerols or phospholipids contained as a natural constituent of cereal grains, or added as a concentrated exogenous supplement via extracted oil from seeds or fruits or rendered animal fat (AAFCO, 2011; NRC, 2012).

Energy is the most expensive constituent of the modern swine diet (Patience, 2012). Therefore, imprecisions in valuing the contribution of dietary fat to the energetics and resulting performance of the growing pig can be potentially costly. Surprisingly, there is little consensus on how to value dietary fat among sources, composition, and chemical characteristics (NRC, 2012). Even the generality that sources of dietary fat that contain a higher unsaturated to saturated fatty acid ratio have greater digestibility than more3 saturated dietary fat sources (Wiseman et al., 1990; Powles et al., 1994) is not consistent (Jorgensen and Fernandez, 2000; Kerr et al. 2009; Kellner et al., 2014).

The objectives of this review are to first provide an overview of the digestion, absorption, and metabolism of dietary fat by the pig. A second objective is to provide a historical progression of techniques employed to determine dietary fats and lipid metabolism differences.

The final objective is to summarize the current digestibility and energy values researchers have assigned to dietary fat sources.

Classification of dietary lipids

Lipids are organic-solvent-soluble, hydrophobic compounds which are molecularly diverse (Palmquist and Jenkins, 2003). Lipids are primarily composed of hydrocarbon structures (i.e., fatty acids and steroids). The primary hydrocarbon structure of lipids, fatty acids are monocarboxylic acids [R-$(CH^2)_n$COO-]. Fatty acids can be classified by their chain length (C:2 to C:24) and or their degree of unsaturation (saturated [SFA = no unsaturated bonds], mono-unsaturated [MUFA = 1 unsaturated bond], and poly-unsaturated [PUFA = 2 or greater unsaturated bonds]). Double bonds can be further classified by their orientation: *cis* (functional groups are on the same side of the fatty acid [carbon] chain) or *trans* (functional groups are on opposite sides of the fatty acid [carbon] chain). Most unsaturated bonds found in nature are cis orientated, but a few *trans* fatty acids are present in nature as a product of bio-hydrogenation processes in the rumen or as a result from industrial processing (Harwood and Scrimgeour, 2007). A fatty acid can also be classified as conjugated (at least 1 pair of double bonds are separated by only 1 single bond; Bee et al., 2008). Except for conjugated linoleic acid, there are no conjugated fatty acids present in nature (Hennessy et al., 2016). In addition, fatty acids can be classified by whether they are esterified or non-esterified to glycerol (NEFA or free fatty acid [FFA]).

Fatty acids can be identified in a multitude of ways (Table 1.1) including: scientific names, common names, systematically, or identifying them by the number of carbons in the fatty acid chain, followed by the number and or location of the double bond(s) (Scrimgeour,

2005; O'Brein, 2009). The chain length and the number and location of double bond(s) of fatty acids can be identified by the International Union of Pure and Applied Chemistry system, which lists the double bonds in relation to the carboxyl carbon (e.g. linolenic acid is Δ9,12,15-18:3), the omega system (also known as the n-minus system), which lists the last double bond in location to the omega carbon (e.g. linolenic acid is C18:3ω6 or C18:3n-6), or the number of carbons and of double bonds only system (e.g. linolenic acid is C18:3; O'Brein, 2009; Kerr et al., 2015). Similar to amino acids, fatty acids can be described as being essential (the pig cannot fully synthesize the required amount needed to sustain proper biological function) or non-essential (NRC, 2012). To date there have only been 2 essential dietary fatty acids determined for the pig (linoleic and linolenic acid; NRC, 2012), so the term non-essential fatty acid is rarely used.

Dietary lipids can be classified by where they are physically located in the diet: intact (contained in the cereal grain) or extracted (added fat to the diet; Kil et al., 2010; Acosta et al., 2015). Extracted dietary lipids can be further classified interchangeably and incorrectly as a fat (animal origin; usually solid at room temperature) or as an oil (vegetable based; can be either solid or liquid at room temperature; AFFCO, 2011). These fat and oil classifications have several incorrect generalities: lipids from fish are called an oil despite being of animal origin, poultry fat can be liquid at room temperature, and vegetable oils can be either solid (coconut and palm) or liquid (corn or soy) at room temperature. The swine industry generally uses dietary fat as the term to identify extracted dietary lipids from any origin. Thus, in the rest of this review and dissertation, dietary fat will be the term that is primarily used to describe added dietary fats and oils.

Digestion of dietary lipids

Dietary lipids are not commonly stored in non-esterified form; instead a majority of them are stored as triacylglycerols or complex lipids such as phospholipids. Some FFAs, diacylglycerols, monoacylglycerols, sterols, and waxes may be present within the diet as well, but in smaller quantities (NRC, 2012). Two major issues arise due to the triacylglycerol storage molecule: lipids are hydrophobic, and the triacylglycerol/complex lipid structure is too large to traverse the intestinal lumen and be absorbed by the enterocyte (Shiau, 1981). Thus, digestive enzymes known as lipases (Table 1.2) are needed to break down the triacylglycerol/complex lipid structure into constituents that are passively absorbed into the enterocyte. Bile salts are needed to overcome the hydrophobic nature of lipids in the lumen of the small intestine.

Lingual and gastric lipase

Dietary lipid digestion starts in the mouth with salivation, mastication, and lingual lipase release (Jones and Rideout, 2012). Lingual lipase (released from the serous gland of the tongue) hydrolyses a FFA from the triacylglycerol structure at the sn-3 (refers to the stereochemcial number of the glycerol backbone) position as the digesta travels from the mouth to the stomach (Hamosh, 1990). Once digesta enters the stomach, gastric lipase continues to hydrolyze dietary triacylglycerides by releasing short chain fatty acids (Hamosh, 1990). Despite hydrolysis by these two lipases, the composition of dietary lipids entering the upper duodenum is still greater than 70% triacylglycerides (Jones and Rideout, 2012). Therefore, the small intestine is the primary location for lipid digestion (Bergstrom and Borgstrom, 1956).

Pancreatic lipase, colipase, and phospholipase A_2

Lipid digestion in the lumen of the small intestine involves 2 key constituents: bile salts and the pancreatic lipase/colipase complex (Jones and Rideout, 2012). Bile salts are constructed from cholesterol in the liver (Langlois et al., 1990). Following construction, bile salts are transported to the gallbladder where they are concentrated (Langlois et al., 1990). The release of bile salts into lumen takes place at the site where emulsion of oil and water occurs (Langlois et al., 1990). The release of bile salts is triggered when circulating levels of cholecystokinin, a peptide hormone, is increased (Langlois et al., 1990). While bile salts are key to lipid digestion and subsequent mixed micelle formation, when released into the lumen of the small intestine they initially create a physical inhibition of pancreatic lipase from coming into contact with lipid droplets (Jones and Rideout, 2012). Colipase reverses the inhibition of bile salts by physically binding to pancreatic lipase (Jones and Rideout, 2012). Once adjoined, the tandem of enzymes can adhere to the outer surface of the lipid droplet, and pancreatic lipase can hydrolyze the ester bond of the triacylglycerol at the sn-1 and sn-3 positions (Borgstrom and Erlanson, 1973). The enzymatic hydrolysis of pancreatic lipase yields 2 FFA and 1 monoacylglycerol with a fatty acid esterified at the sn-2 position. The enzymatic activity of pancreatic lipase is rapid and produces FFA and monoacylglycerols at a rate greater than subsequent micelle incorporation (Vandermeers et al., 1974). Phospholipids are resistant to hydrolysis via pancreatic lipase (Borgstrom, 1980). Therefore, phospholipids undergo enzymatic digestion via phospholipase A_2 (Borgstrom, 1980). Phospholipase A_2 hydrolyzes the fatty acid from the sn-2 position (Borgstrom, 1980). The yield of phospholipase A_2 activity is lysophosphoglycerides and FFA (Borgstrom, 1980). Colipase then shuttles the recently hydrolyzed FFA and monoacylglycerides from the lipid droplets to the forming micelle (Jones and Rideout, 2012).

Micelle formation

Once pancreatic lipase activity begins, complexes of lipid material that are soluble in water (unlike dietary lipids pre-digestion that were insoluble in water) called micelles begin to form (Shiau, 1981). Micellar formation occurs from the actions of bile salts and phospholipids, which are secreted in bile from the gallbladder (Jones and Rideout, 2012). Bile salts have a polar end located outward the water milieu of the digesta and intestinal lumen, and a nonpolar end located towards the center of the micelle (Zwicker and Agellon, 2013). The orientation of bile salts along with phospholipids creates a micelle conglomeration with a center that is hydrophobic and edges which are hydrophilic (Jones and Rideout, 2012). After micelle formation and subsequent saturation of lipid products, the micelle travels across the lumen to the unstirred water layer next to the apical membrane of the enterocyte (Jones and Rideout, 2012). Micelles solve the problem of lipids being hydrophobic in aqueous environments (Jones and Rideout, 2012). Thus, micelles allow lipid material (now contained in a micelle) to easily traverse the unstirred water layer (Jones and Rideout, 2012). Micelles concentrate FFA, monoacylglycerols, and other lipid materials at the absorptive surface of the enterocyte by 100 to 1000 times (Westergaard and Dietschy, 1976). A simple overview of dietary fat digestion and absorption just described is depicted in Figure 1.1 (adapted from Jones and Rideout, 2012).

Endogenous losses of dietary fat digestion

Endogenous losses of digestion are nutrients that appear in digesta or in feces that are not of dietary origin (NRC, 2012). Endogenous losses can be estimated as basal losses (non-diet specific losses, to estimate standardized digestibility) or diet specific losses (to estimate true

digestibility; Stein et al., 2007; NRC, 2012). Endogenous losses of dietary fat digestion may originate from several sources throughout the gastrointestinal tract via sloughed intestinal cells, microbial mass, and intestinal secretions (Kil et al., 2010). This multitude of sources makes estimating the endogenous losses of dietary fat digestion difficult and creates differences in the estimation of endogenous losses between the terminal ileum and the end of the large intestine (Kil et al., 2010; Gutierrez et al., 2016).

Absorption of dietary lipids

Passive transport

Due to a gradient created by concentrating lipid material (FFA and 2-monoglycerids) in a micelle, lipid constituents can passively diffuse into the enterocyte by a non-energy dependent process (Johnston and Borgstrom, 1964). Micelles maintain equilibrium with other micelles due to the churning action and structure of the intestine, causing nearly constant contact among the epithelium, micelles, and lipid droplets (Jones and Rideout, 2012). During this constant contact, lipid constituents are partitioned from highly populated micelles to less populated micelles (Jones and Rideout, 2012). This constant partitioning causes micelles to equally acquire and distribute lipid constituents, so that the ultimate factor that limits lipid digestion in the small intestine is micelle saturation (Jones and Rideout, 2012). Shuttling of lipid constituents from micelles across the unstirred water layer is a chain reaction that requires a lower cellular concentration of lipids at the enterocyte (Jones and Rideout, 2012). It has been theorized that intestinal fatty acid binding proteins increase fatty acid uptake by binding to FFA and then entrapping them within the vicinity of the apical membrane (Stremmel et al., 2001). Bile salts are efficiently recycled via absorption in the lower ileum (via assistance from ileal lipid binding

proteins) and transported back to the liver for reuse in subsequent lipid digestion (Zwicker and Agellon, 2013).

Active transport

Evidence also supports the idea of a carrier dependent absorption process when lipid concentrations in the lumen of the small intestine are low (Chow and Hollander, 1979). The theory about active transport of long chain fatty acids is that it involves intestinal fatty acid binding proteins and/or a fatty acid translocase (Minich et al., 1997). The dual mechanism for lipid absorption (passive and active transport) is theorized to maintain required essential fatty acid levels (linoleic and linolenic acid) when dietary lipid intake is low, but it is currently unknown how important carrier mediated transportation is when dietary lipid intake of the pig is normal or high (Kindel et al., 2010).

Non-small intestinal fatty acid absorption

Not all fatty acid absorption occurs in the small intestine. Short and medium chain fatty acids can be passively absorbed through the gastric mucosa after hydrolysis via lingual and gastric lipase (Lemarie et al., 2016). Furthermore, there is evidence that short and medium chain fatty acids can be passively absorbed and metabolized by colonic epithelium (Jorgensen et al., 2001). How substantial the rate of fatty acid absorption is (if any) in gastric and colonic tissues in the grow-finish pig is currently unknown.

Circulation and uptake of fatty acids by target tissues

Re-esterification and chylomicron construction

Once diffusion into the enterocyte has occurred, long chain fatty acids (≥ C14:0) are re-esterified in the endoplasmic reticulum via the glycerol-3-phosphate pathway or the monoacylglycerol pathway (Cunningham and Leat, 1969). Once re-esterified into a triacylglyceride, multiple triglycerides and cholesterol esters are packaged into chylomicrons (Sabesin and Frase, 1977). Chylomicrons are composed of 80 to 95% triacyglcerides, 2 to 7% cholesterol, and 3 to 9% phospholipids (Jones and Rideout, 2012). The exterior surface of a chylomicron has a phospholipid bi-layer and apolipoproteins which increase solubility and enzymatic recognition (Shiau, 1981). The chylomicron enters the circulatory system via the lymphatic system at the thoracic duct (Shiau, 1981).

Circulation

Not all recently digested dietary fatty acids are circulated via a chylomicron; instead, short and medium chain fatty acids (≤ C12:0) can be circulated in non-esterified from while bound to albumin (Jones and Rideout, 2012). Unlike their longer chain counterparts, which are circulated to target tissues like adipose and muscle, these short chain fatty acids after absorption are directed to the liver via the portal vein (Bach and Babayan, 1982; Foufelle, 1992).

Past this initial circulation and uptake of recently digested dietary fatty acids, mobilized fatty acids can be transported by lipoproteins (Table 1.3). Lipoproteins solve the problem of lipids being hydrophobic and provide an advantage to increase particle solubility, concentration of lipids, and recognition of enzymes and receptors (Jones and Rideout, 2012).

Uptake by target tissues

Once the chylomicron is circulated, the lipid products within can be stored in the adipocyte, or oxidized by myofibers and other cells (Jones and Rideout, 2012). If the concentration of insulin is elevated, chylomicrons will be directed to adipocytes for storage (Wang and Eckel, 2009). Insulin moderates stimulation of adipocyte lipoprotein lipase, but the isoform of lipoprotein lipase in the muscle cell is not stimulated by insulin (Wang and Eckel, 2009). When insulin concentration is high, lipoprotein lipase will be expressed in the capillary lumen of the adipocyte to process triglyceride-rich chylomicrons and other lipoproteins (Wang and Eckel, 2009). Fatty acids will be passively diffused individually into the adipocyte (via fatty acid binding proteins) and then re-esterified for storage as a triacylglyceride in the adipocyte (Jones and Rideout, 2012).

Post-absorptive metabolism of lipids

De novo lipogenesis

Nearly all de novo lipogenesis in the pig takes place in adipose tissue (Figure 1.2), unlike in humans or rodents, where nearly all lipogenesis takes place in the liver (O'Hea and Leveille, 1969). Excess acetyl Co-A is the substrate utilized in de novo lipogenesis (Lawes and Gilbert, 1886). Excess acetyl Co-A is created when glucose or other monosaccharides are not needed to fuel tissues within the pig. The creation of excess acetyl Co-A and the transfer of the substrate of lipogenesis into the cytosol of the adipocyte is complex, involving many enzymes and regulators.

The lipogenesis process in the porcine adipocyte starts when glucose is converted to pyruvate under a series of reactions known as glycolysis in the cytoplasm of the cell. Pyruvate

then has four possible fates: conversion to alanine, oxaloacetate, lactate or acetyl Co-A (Heckler, 1997). The conversion of pyruvate to acetyl Co-A requires Co-A, NAD+, and the enzyme pyruvate dehydrogenase; this reaction creates acetyl Co-A and NADH plus a hydrogen ion (Denton et al., 1975). Acetyl Co-A then has multiple possible fates; if energy is needed by the pig, it can enter the TCA cycle for ATP production. Acetyl Co-A can also be used in the synthesis of various amino acids, or transported to the liver to produce ketone bodies (Akram, 2013). However, when the energy needs of tissues within the pig are met, excess acetyl Co-A is used to initiate lipogenesis.

Formation of acetyl Co-A in the mitochondria creates a problem. Acetyl Co-A is unable to pass through the mitochondrial membrane to the cytosol, which is where lipogenesis occurs. Therefore, acetyl Co-A must be converted to citrate in the mitochondria. Due to its structure, citrate can transverse the membrane bi-layer of the mitochondria and move into the cytosol (Remington, 1992). The enzyme citrate synthase converts acetyl Co-A to citrate. Once citrate has reached the cytoplasm through the tricarboxylate transporter, it must reconvert to acetyl Co-A before lipogenesis can proceed (Remington, 1992). High concentrations of citrate in the cytosol of the adipocyte will activate ATP-citrate lyase, an enzyme that cleaves oxaloacetate from citrate to recreate acetyl Co-A.

In the process of transferring acetyl Co-A into the cytosol, oxaloacetate is now also present. Due to the composition of oxaloacetate, it cannot re-enter the mitochondrial matrix (Ackrell, 1974). Therefore, oxaloacetate must be converted into malate via malate dehydrogenase, and then transferred via the same tricarboxylate transporter through which citrate entered the cytosol in an exchange process that is stimulated by increased citrate in the mitochondria, creating a concentration gradient (Danis and Farkas, 2009).

What happens if there is not enough citrate present in the mitochondria for a high enough concentration gradient to occur? Instead of malate being exchanged for citrate, malate can be oxidized to pyruvate, which in turn produces an NADPH that will be utilized later in de novo lipogenesis (Flatt, 1970). Pyruvate can then be exchanged with a hydrogen ion back to the mitochondria via the pyruvate transporter (Flatt, 1970).

The rate limiting step in de novo lipogenesis occurs right after acetyl Co-A has entered the cytoplasm. Formation of malonyl Co-A is a two-pronged reaction that involves the carboxylation of biotin (involving ATP) and the transfer of the carboxyl group to acetyl Co-A to form malonyl Co-A via acetyl Co-A carboxylase (Lane et al., 1974). Acetyl Co-A carboxylase is the rate limiting enzyme, regulated by the concentration of citrate and by the phosphorylation that governs allosteric sensitivity (Lane et al. 1974). The malonyl Co-A formation is a two-step reaction that not only limits the rate of de novo lipogenesis, but also creates the irreversible process of de novo lipogenesis in the adipocyte.

Fatty acid synthase adds 2 carbon units with malonyl Co-A as the donor in succession until terminal thioesterase (which is sterically activated by palmitic acid, a saturated fatty acid containing 16 carbons) releases the completed fatty acid (Clarke, 1993). The enzyme complex is a homodimer with two catalytic centers that exerts both transcriptional and post-transcriptional control (Clarke, 1993). Fatty acid synthase is not sensitive to phosphorylation, but is highly sensitive to dietary fat intake (Allee et al., 1971; Smith et al., 1996).

The net reaction of a synthesized palmitic acid is: 1 acetyl Co-A + 7 maloynl Co-A + 14 NADPH + 14 H+ = 1 palmitate (C16:0) + 8 Co-A + 7 CO_2 + 14 NADP+ + 6 H_2O. While palmitic acid is most widely used in explaining de novo lipogenesis, Kloareg et al., (2007) found that only 33% percent of de novo synthesized fat was deposited as palmitic acid (C16:0). Nearly

66% of palmitic acid was elongated to steric acid (C18:0), and more than 70% of the elongated C18:0 fatty acids were desaturated by delta-9 desaturase at the omega-9 position to produce oleic acid (C18:1). Therefore, Kloareg et al., (2007) showed that the fatty acid most likely to be produced by de novo lipogenesis in the pig is oleic acid and not palmitic acid.

Lipolysis

Lipolysis (Figure 1.3) is the process of breaking down a triacylglycerol molecule in storage into 3 NEFA and 1 molecule of glycerol. Lipolysis is required to mobilize fat for use in other tissues, as fat in the form of triacylglycerols cannot exit the adipocyte, due to its size. Enser (1984) reported that a diet containing sufficient energy to meet the needs of the growing pig needed to reduce its reliance on lipolysis. Because lipolysis in the adipocyte is continuous, released fatty acids are re-esterified and remain in the adipocyte when energy supply is adequate (Mears and Mendel, 1974). Thus, fatty acid turnover will not be a significant factor under normal growing conditions in altering the fatty acid composition and resulting pork fat quality.

However, if energy balance was negatively affected via an immune challenge, fasting, or another insult to the pig's energy balance, theoretically lipolysis could be a significant factor in determining pork fat quality (Bee et al., 2002). Raclot et al. (1995) reported in fasting rats that the mobilization of fatty acids depended on the location of the fatty acid on the glycerol back bone. In pigs it has been reported that extreme changes in energy intake, combined with a change in dietary fat source, alter the composition of deposited pork fat (Wood et al., 1985, 1986), and that the pork fat becomes more unsaturated as dietary energy intake decreases (Bee et al., 2002).

Lipolysis of triglycerides stored in the adipocyte of the pig starts with adipose triacylglyceride lipase (Jenkins et al., 2004). Adipose triacylglyceride lipase cleaves the first ester bond of the triacylglyceride to create a FFA and a diacylglyceride. The second ester bond of the initial triacylglyceride to be hydrolyzed is created by hormone sensitive lipase, which cleaves the second fatty acid from the newly created diacylglyceride, creating 2 FFA and a monoacylglyceride (Young and Zechner, 2013; Vaughan et al., 1964). The third enzyme, called monoacylglyceride lipase, hydrolyzes the final ester bond, which cleaves off the final fatty acid from the glycerol backbone (Young and Zechner, 2013). These 3 enzymatic reactions of lipolysis (hydrolyze the 3 ester bonds of a triacylglycerol molecule) result in 3 non-esterified fatty acids and 1 molecule of glycerol. Each of these enzymes, as well as perilipin (the coding around the lipid droplet in the adipocyte), are activated by phosphorylation via protein kinase A, which is activated by cyclic adenosine monophosphate (Young and Zechner, 2013). Once enzymatically cleaved, the recently hydrolyzed non-esterified fatty acids will bind to fatty acid binding proteins until they reach the endothelial barrier, where they are then bound to albumin and transported into circulation (Young and Zechner, 2013).

Beta-oxidation

Fatty acids are superior to other macronutrients because of their high proportion of carbon-hydrogen bonds (Jones and Rideout, 2012). After hydrolysis via lipoprotein lipase (located on the exterior of the myocyte) fatty acids enter the cytoplasm (Jones and Rideout, 2012). However fatty acids cannot transverse the mitochondrial membrane without undergoing activation to fatty acyl-CoA, and long chain fatty acids must be bound to carnitine via carnitine palmitoyltransferase-mediated binding (Jones and Rideout, 2012; Watt and Hoy, 2012). After

entering the mitochondria, carnitine is efficiently recycled back to cytoplasm, and fatty acids are reactivated by CoA (Jones and Rideout, 2012). The 2 beta carbon atoms of the acyl chain undergo degradation via four distinct steps: dehydrogenation (removal of hydrogen); hydrogenation (addition of water); dehydrogenation (removal of hydrogens); and cleavage (Jones and Rideout, 2012; Watt and Hoy, 2012). If the 2 beta carbons are bound doubly, creating an unsaturated bond, the initial dehydrogenation reaction does not occur (Jones and Rideout, 2012). This cycle of oxidizing the 2 beta carbons is repeated until the acyl chain is completely oxidized (Jones and Rideout, 2012).

Techniques used to evaluate the digestibility of dietary fat

Digestion measured via total tract or ileal cannulation

Digestibility of dietary lipids was first differentiated in humans over a century ago (Atwater, 1900; Maynard 1944). Since then, total dietary lipid digestion has been measured in pigs primarily over the entire gastrointestinal tract (Jorgensen et al., 1992). The apparent digestibility of total dietary lipids was generally accepted to be similar when measured over the total tract through analysis of fecal matter versus digesta, collected through a cannula inserted at the terminal ileum (Jorgensen et al., 1992). This, however, is no longer an accepted conclusion as Duran-Montge et al. (2007), Kil et al. (2010), and Kim et al. (2013) showed that ileal digestibility was more effective than digestibility measured over the total tract, due to losses of endogenous and microbial fat in the hindgut. Due to microbial hydrogenation of unsaturated fatty acids in the cecum and large intestine (Bayley and Lewis 1965, Jorgensen et al., 1992), uptake of individual fatty acids must be measured at the terminal ileum (Jorgensen et al., 1992; Jorgensen et al., 2000). Measurement of apparent digestibility of dietary fat or fatty acids in

other monogastric species is typically done over the total tract (Tou et al., 2011; Geng et al., 2012). The apparent total tract digestibility of fat can be determined by total collection (total input – total output) or by employing a digestibility marker via the index method (i.e., chromic oxide or titanium dioxide; Adeola, 2001). The advantage of the index/marker method is that it does not require pigs to be housed in metabolism stalls, so the quantity of fecal matter collected is dramatically decreased (Adeola, 2001). Since it is difficult to collect 100% of digesta when using a T cannula at the terminal ileum, in most instances a digestibility marker is used.

Endogenous losses of fat digestion

Endogenous losses of digestion can be estimated as basal losses (non-diet specific losses) to estimate standardized digestibility or as diet specific losses (to estimate true digestibility; Stein et al., 2007; NRC, 2012). Total endogenous losses of fat digestion have been determined utilizing the regression method (via feeding increasing levels of dietary fat; Jorgensen et al., 1993). This method uses the y-intercept to determine the diet specific endogenous losses of fat digestion (for true total tract digestibly of AEE). Previous estimates of EFL (Table 1.4) over the total tract via the regression method have ranged from 22.4 (Jorgensen and Fernandez, 2000) to 3.8 (Kil et al., 2010) g/kg of dry matter intake. To date there is no published estimation of the basal losses of the endogenous losses of dietary fat via feeding a fat-free diet in pigs.

Collection of digesta post-harvest

Recently, Tancharoenrat et al. (2014) used a serial slaughter approach in broilers to measure the disappearance of fat and fatty acids along differentiated sections of the

gastrointestinal tract. The disappearance was then corrected for endogenous losses from the collection of digesta and bile from the gall bladder when the broilers were fed a fat-free diet.

In vitro digestion of dietary lipids

In vitro digestion of dietary lipids has been unable to mimic *in vivo* digestion of lipids by the pig (Wang et al, 2012). Wang et al. (2012) discovered that the *in vitro* model adapted from Boisen and Fernandez (1997) did not optimize fat digestion through added lipases and did not simulate microbial hydrogenation in the hindgut.

Techniques used to estimate the energy value of dietary fat

Dietary energy systems

Assigning energy values to dietary fat sources requires an understanding of each energy system. Gross energy (GE) measures energy via combustion in an oxygen enriched environment to ascertain the total quantity of energy contained in a lipid sample. Digestible energy (DE) corrects GE for the portion of energy that is contained in fecal matter. Metabolizable energy (ME) corrects DE for the loss of energy contained in urine and gases. Net energy (NE) corrects ME by accounting for the metabolic cost of converting energy into forms that can be utilized by the pig, also known as the heat increment (Patience, 2012).

Estimation of DE

The overwhelming majority of estimated energy values of dietary fat sources are on a DE basis (NRC, 2012). Dietary fat DE is defined as the GE of dietary fat minus the energy in fecal matter. The DE can be determined via the total collection to index marker methods described

previously (Adeola, 2001). There are two issues with the DE system. First the DE system does not account for losses of energy in urine or gasses or accounts for energy lost as heat. Secondly, some of energy contained in fecal matter is not of dietary origin, but is of endogenous origin.

Research compiled in Powles et al. (1995) via Wiseman et al. (1990) and Powels et al. (1993 and 1994) was used to estimate the current NRC (2012) DE content using the equation: DE, kcal/kg = {36.898 – [(0.005 × FFA (free fatty acid), g/kg) – (7.330 × $\exp^{-0.906 \times \text{U:S (unsaturated:saturated fatty acid ratio)}}$]} / 4.184. These series of experiments used dietary fat sources that ranged from 0.66 to 15.67 U:S and 0.8 to 81.8% FFA level. These sources were blended to create a range of data points (Powles et al., 1995). However, these experiments included dietary fat sources with primarily 16 or 18 carbon chain length fatty acids; thus the accuracy of the equation was unknown for shorter fatty acid chain sources (i.e., coconut oil) or longer fatty acid chain sources (i.e. fish oil; NRC, 2012).

Estimation of NE via indirect calorimetry

Oxygen consumption and carbon dioxide output are highly correlated to the production of heat (Adeola, 2001). Thus, the estimation of heat production can be quantified by the flow of oxygen and carbon dioxide (Adeola, 2001). As described earlier, NE is defined by correcting ME for energy losses via heat increment (Patience, 2012). Therefore, by estimating losses of heat using the net flow of carbon dioxide, oxygen, and methane in a respiration chamber, one can estimate the NE of an ingredient or diet.

The current NRC (2012) estimate of NE is derived from the DE values generated using the Powles et al. (1995) equation, which are then converted from DE to ME by a 98% conversion and from ME to NE by an 88% conversion. These percentages are obtained from indirect

calorimetry data reported by van Milgen et al. (2001), in an experiment with 5 pigs (~60 kg BW), which determined that 7% vegetable oil has 8.099 Mcal/kg of DE, 7.977 Mcal/kg of ME and 7.107 Mcal/kg of NE. To date these are the only data available to estimate the NE of dietary fat using indirect calorimetry.

Estimation of NE via comparative slaughter technique

Another way to estimate the NE of dietary fat sources in growing and finishing pigs (Table 1.5) is through serial slaughter (Galloway and Ewan, 1989; Kil et al., 2011). Energy is determined by evaluating pigs (a start and final group) that are slaughtered, ground, dried, homogenized and oxidized via bomb calorimetry. The difference between the total carcass energy in the start group and the final group is used to determine the NE of the feed or ingredient (Adeola, 2001).

Galloway and Ewan (1989) estimated the DE, ME, and NE of TAL at 8.24, 7.88, and 4.18 Mcal/kg respectively, in nursery pigs. Kil et al. (2011) estimated the NE of soybean oil and choice white grease at 4.68 and 5.90 Mcal/kg respectively in growing and finishing pigs. These estimates are considerably lower than the estimates reported by Boyd et al. (2015) and lower than current NRC (2012) and INRA (Sauvant et al., 2004) estimates. The discrepancy in the results using these different methodologies lies in the efficiency of ME for NE of lipids, which is assumed to be extremely efficient (Noblet et al., 1993; Jorgensen et al., 1996; van Milgen et al., 2001; Boyd et al., 2015), but in these comparative slaughter experiments the efficiency of ME to NE was lower than expected (Kil et al., 2011). Furthermore, the comparative slaughter method is plagued with a high degree of error, much greater than obtained using indirect calorimetry.

Growth assay calibration

Growth assays can be utilized to validate NE estimates using gain:feed as the primary outcome (Boyd et al., 2015). Experiments can be designed to achieve constant gain:feed or differing gain:feed. In ether instance, if the expected gain:feed differs from the theoretical, the NE value of the diet, and thus the fat source, is adjusted accordingly (Figure 1.4).

A calibration of the NRC (2012) NE estimate of dietary fat sources was completed in a commercial scale growth-assay by testing choice white grease and employing a diluent (bentonite, fine washed sand), which determined that the NE was 8.059 Mcal/kg (BW 38 to 67 kg) and 8.502 (BW 79 to 107 kg), 10% and 14% respectively greater than the NRC (2012) estimate (Boyd et al., 2015).

Scope of dietary fat sources tested

One of the primary issues with evaluating dietary fat is that the amount of fat that can be included in the diet is limited (Carter, 2010). Another issue is the diverse chemical structure among dietary fat sources. The vast majority of experiments that have evaluated dietary fat in pigs (experiments with humans and rodents have been more diverse) have been of sources that are 16 to 18 carbons of length and have an U:S ranging from 1 to 6 (NRC, 2012). This limited range of chain length and degree of unsaturation is due to experiments that evaluate products that are most likely to be employed in commercial diets (i.e., choice white grease, animal-vegetable blend, or corn oil). The NRC (2012) clearly points out that the accuracy of current prediction equations of dietary fat is unknown for sources composed of medium chain fatty acid or with a high degree of PUFA.

Techniques used to evaluate changes in lipid metabolism

To quantify the effect of changes in dietary fat on enzymes involved in lipid metabolism in the pig, analysis can be performed on the abundance of the message for protein translation, the activity of the enzyme, or the abundance of the protein itself. Additionally, the pig has a superior ability among domesticated livestock species to store excess energy as fat (Wood et al., 2008). Thus, understanding how dietary fat intake alters the rate of de novo lipogenesis is important in accurately predicting the pig's response to dietary fat.

Messenger RNA abundance

Proteins (i.e., enzymes) are transcribed and then translated from a deoxyribonucleic acid (DNA) origin which is located in the nucleus of the cell. Messenger ribonucleic acid (mRNA) is transcribed from DNA via RNA polymerase II with the functional objective of "messaging" genetic information from the DNA to the ribosome (a cellular organelle which translates the functional protein for use by the cell).

Polymerase chain reaction was developed by Kary Mullis in 1983 (awarded the 1993 Noble Prize for chemistry), and has been used extensively ever since (Bartlett and Stirling, 2003). Quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction, can be used to quantify the abundance of mRNA present in tissue (Wang et al., 1989). The quantification of mRNA abundance starts with isolating mRNA from a specific tissue sample (i.e., adipose or liver), followed by the creation of complementary DNA (cDNA) via reverse transcription of mRNA (Peirson and Bulter, 2007). A dye (i.e., SYBR Green) is then added to a solution that includes cDNA and primers (forward and reverse) of the gene to be investigated (Perison and Bulter, 2007). The qPCR mix just described is then rapidly heated and

cooled (thermal cycle). A thermal cycle consists of 3 stages: separation of the nucleic acid double chain (at ~95°C), binding of the forward and reverse primers with the DNA template (at ~55°C), polymerization (at ~70°C), resulting in fluorescence (as the dye only fluoresces when bound to double stranded DNA [the qPCR product]; Perison and Butler, 2007). The intensity of the fluorescence is then measured with a detector (Tichopad et al., 2003). The most common reported parameter of qPCR is cycle threshold (Ct), which is the number of thermal cycles required for the intensity of the fluorescents to cross a given value (i.e., 10 standard deviations above the baseline; Tichopad et al., 2003).

Enzyme activity

The objective of an enzyme activity assay is to quantify the amount of enzyme present, allowing for the comparison of enzyme activity between or among samples (Scopes, 2002). However, enzyme activity is measured *in vitro* and often cannot replicate what is present *in vivo* (i.e. similar substrate concentrations, pH, and temperature; Scopes, 2002). Additionally, most enzyme activity *in vivo* is continuous and decreases or increases due to a physiological need, while enzyme activity measured *in vitro* quantifies the initial rate of substrate utilization with no products present (a scenario that is highly unlikely to occur *in vivo*; Scopes, 2002).

The basic goal of each enzyme activity assay is to quantify how much substrate has been used or how much product has been formed over a known period of time (Scopes, 2002). Assays can be performed by measuring the substrate or product itself via chemical quantification or a separation method (i.e., high-performance liquid chromatography) or by using absorbance or fluorescence (Scopes, 2002).

Protein abundance

A series of methodologies (2-dimensional electrophoresis and mass spectrometry with validation of a western blot analysis) can determine actual protein (i.e., enzyme) abundance (Greenbaum et al., 2003; Di Luca et al., 2013). Two-dimensional electrophoresis was first introduced by O'Farrell (1975) and Klose (1975). The 2 dimensions of this electrophoresis (motion of dispersed particles relative to a fluid while under the influence of a uniform electric field) process are: separation of proteins linearly according to their isoelectric point and separation of proteins according to their molecular mass (Greenbaum et al., 2003). Images are created via staining by comparing two samples, plus a pooled reference sample per gel (Pearce et al., 2015). Using imaging software and statistical analysis, "spots" are identified as being differentially abundant (Pearce et al., 2015). These "spots" are then identified for a specific protein/peptides via mass spectrometry (Di Luca et al., 2013). Validation of these 2-dimensional electrophoresis data can be carried out by western blot analysis. Western blot analysis was first introduced by Towbin et al. (1979) and uses an antibody to specifically detect a target protein. If the specific protein targeted is present, a stained band on the blot will be present in post-gel electrophoresis (Mahmood and Yang, 2012).

Rate of de novo lipogenesis

Measuring changes in the rate of de novo lipogenesis in the pig due to management or other changes requires a baseline value. Many publications have defined the lipid deposition rate in the pig under varying environments and dietary intakes (NRC, 2012). However, very few researchers have attempted to quantify lipid deposition from dietary carbohydrates or fat. Previous findings have shown varied results due to differences in age, weight, genetics, diet

content, and energy intake. Kloareg et al. (2007) reported that a 65 kg pig housed in thermoneutral conditions and with ad libitum access to feed deposited 175 grams per day of fat generated de novo, which was most of the 209 grams per day of total fat deposited. Allee et al. (1971) used radio-labeled glucose for 45 days to measure the de novo deposition rate for pigs with a 95 kg market weight, fed 24% crude protein and 1% added dietary fat; they reported that the fat deposition rate under these conditions was 369 g/d. Lizardo et al., (2002) used a model to predict pork fat composition by assuming the de novo lipogenesis accounted for 80% of total lipid deposition for finishing pigs; a recent review concluded that the total lipid deposition rate for a 70 kg pig was 294 g/d (Patience, 2012). The Lizardo model, however, was hampered by insufficient data relating diet intake and lipid deposition. Unfortunately, none of these results quantify de novo lipogenesis rates above 100 kg. Considering that maximum lipid deposition occurs later during the finishing stage, and that pigs are currently harvested at 130 kg, these values may be inaccurate when defining the balance between the deposition of preformed fatty acid and de novo synthesized fatty acids at or near current market weight.

Others have attempted to quantify de novo lipid deposition via changes measured in the fatty acid composition deposited or the incorporation of glucose into neutral lipids (Mourot et al., 1994; Smith et al., 1996). These attempts do show that lipogenesis is altered, but they are not precise enough to quantify an actual deposition rate of de novo generated fatty acids. Considering all dietary interventions and environmental treatments reported above, the de novo synthesis and deposition rate of fat in the pig can range from nearly 100 g/d to 300 g/d for the growing pig weighing from 50 to 120 kg.

Effects due to the chemical structure of dietary fat

Digestion and absorption

Chain length, degree of unsaturation, esterification with glycerol (i.e. FFA or triacylglycerol), age/size of the pig, and the interactions among these have impacts on how efficiently, where, and how dietary fat is digested from the mouth to terminal ileum. Over the past 60 years a multitude of digestibility studies have been conducted in pigs. Table 1.6 shows a chronological order of these studies and their key findings.

The cumulative findings of these studies (Table 1.6) does not present a unanimous consensus nor a clear picture of how digestion and absorption differs among dietary fat sources. However, these data as a whole, combined with known biochemistry, do clarify some of the impacts of chemical composition on the digestibility of dietary fat. To start, lipids are hydrophobic, and it's currently thought that triacylglycrides are less hydrophobic than FFA and PUFA are less hydrophobic than MUFA, which are less hydrophobic than SFA (Liu et al., 2015). Wiseman (1990) additionally suggested that FFA compared to fatty acids esterified to glycerol could suppress secretion of bile salts. Thus, increased dietary fat content of FFA will decrease the digestibility of dietary fat (Mendoza and van Heugten, 2014), but these affects will be less if the dietary fat source is highly unsaturated (Rosero et al., 2015), and as the pig increases in age/size (Powles et al., 1995), due to being less reliant on bile slats for emulsification and micelle incorporation. If FFA are not incorporated into the micelle, it is currently assumed that they cannot transverse the unstirred water layer, which blocks absorption into enterocytes, and therefore they are passed into the large intestine (Kerr et al., 2015).

It is currently theorized that pancreatic lipase has greater affinity for PUFA than SFA (Birk et al., 2004; Goncharova et al., 2014). Thus in theory, dietary fat sources with unsaturated

fatty acids in the Sn-1 and Sn-3 positions of the triacylglyceride, would be more efficiently digested than sources that have SFA in those positions (Bracco, 1994). When incorporating lipid material into the structure, some evidence supports the idea that micelles have a higher affinity for PUFA and saturated monoacylglyerols (Hofmann and Mekhijian, 1973; Bracco, 1994). As previously mentioned, unsaturated fatty acids are thought to be less hydrophobic than SFA (Liu et al., 2015). This chemical property may aid incorporation of unsaturated fatty acids into the micelle with greater efficiency than SFA. It is also theorized that long chain unsaturated fatty acids can be transported across the lipid bilayer of the enterocyte via both passive and active transport (Minich et al., 1997; Kindel et al., 2010). To add to the complexity of determining the digestibility of a fatty acid based on its degree of unsaturation, Tacharoenrat et al. (2014) recently found in broilers that linoleic acid (C18:2) is absorbed by enterocytes throughout the entire small intestine, while SFA and MUFA are not absorbed until they reach the jejunum. This may explain why there is a tendency for the digestibility of dietary fat sources to increase as the degree of unsaturation of the dietary fat sources increases. The positive impact of increased unsaturated fatty acid to SFA on digestibility of dietary fat is supported by Cera et al. (1988), Powles et al. (1995), and Rosero et al. (2015). It differs with Jorgensen et al. (2000) and Kil et al. (2011) who reported no difference among sources differing in degree of unsaturation and Mendoza and van Heugten (2014) who reported a decrease of digestibility as the iodine value of the dietary fat source increased.

Medium chain fatty acids (< C14:0) can be cleaved from the triglyceride molecule by lingual, gastric, and pancreatic lipases (Hamosh, 1990). Additionally, it is theorized that short and medium chain fatty acids can be passively absorbed through the gastric mucosa (Lemarie et al., 2016). The increased enzymatic hydrolysis potential and absorptive surface area along the

digestive tract that short chain fatty acids have compared to long chain fatty acids may explain the reports by Lloyd et al. (1957) and Cera et al. (1989 and 1990) showing increased digestibility of coconut oil versus corn or soybean oil.

Fully hydrogenated fat was investigated as a way to improve carcass iodine values or reduce the unsaturated fatty acid content of deposited pork fat (Averette Gatlin, 2005). It was found that the digestibility of hydrogenated tallow or choice white grease is nearly 0 (Tullis and Whittemore, 1980; Averette Gatlin, 2005). The extent that moisture, insoluble, unsaponifiables, non-eluatable material and oxidized lipids contained within dietary fat sources have on digestibility in pigs has not been well established (Kerr et al., 2015). Clearly, differentiating the digestibility of dietary fat sources based on analyzed composition has proven to be difficult and needs further clarification (NRC, 2012).

Re-esterification, circulation, and uptake

The difference among fatty acids from absorption by the enterocyte to uptake by the target tissue is due to chain length. Medium chain fatty acids (< C14:0) once absorbed into the enterocyte enter portal capillaries and traverse via the portal vain to the liver (Odle, 1997). In contrast, long chain fatty acids (≥ C14:0) are re-esterified, packaged into chylomicrons, directed through the lymphatic system, and then circulated to target peripheral tissues such as adipose and muscle (Bach and Babayan, 1982; Odle, 1997). Thus, when compared to long chain fatty acids, the exposure of medium chain fatty acids (from a dietary fat source such as coconut oil) in liver is much greater than adipose tissue (Foufelle, 1992; Odle, 1997).

Lipogenesis

It is generally accepted that increasing the level of dietary fat will suppress fatty acid synthase (a multi-faceted enzyme that synthesizes palmitate (C16:0) from malonyl CoA in the cytosol of the adipocyte [Beld et al., 2015]) and the rate of de novo lipogenesis in adipose tissue (Allee et al., 1971; Smith et al., 1996). Thus, the fatty acid profile of the diet is reflected more in the fatty acid profile of depot fat (Kellner et al., 2014).

Less clear is the effect of dietary fat source and the degree of unsaturation of the source on de novo lipogenesis. Allee et al. (1971) found that the suppression of lipogenesis did not differ in growing pigs fed either 10% corn oil (unsaturated source) or tallow (saturated source). Later, Smith et al. (1996) reported a greater rate of lipogenesis in cultured porcine adipocytes with a linoleic acid (C18:2) enriched diet versus an oleic acid (C18:0) enriched diet. Kouba and Mourot (1998) reported greater mRNA abundance of acetyl-CoA carboxylase and fatty acid synthase in pigs fed corn oil rather than tallow. The explanation for SFA being a more potent inhibitor of de novo lipogenesis in adipose tissue than omega-6 fatty acids (linoleic acid [C18:2] in particular) is that in pigs, dietary fatty acids are largely unmodified in composition (chain length and degree of unsaturation) from ingestion to deposition (Ellis and Isbell, 1926; Kellner et al., 2014). De novo synthesized fatty acids are SFA (palmitic acid [C16:0] and stearic acid [C18:0]) or MUFA (palmitoleic acid [C16:1] or oleic acid [C18:1]; Kloareg et al., 2007). Thus, if the pig consumes and deposits SFA of dietary origin, there is less need for the adipocyte to synthesize fatty acids of similar chemical structure (C16:0, C16:1, C18:0, and C18:1). However, if the pig consumes and deposits omega-6 fatty acids (C18:2) of dietary origin, the negative effect on de novo lipogenesis in the adipocyte does not apply to the same the degree.

Lipolysis

Koch et al. (1968) first suggested that linoleic acid and linolenic acid were maintained in the subcutaneous adipose tissue as a reservoir of essential fatty acids, and these fatty acids are retained in adipose tissue greater than other non-essential fatty acids during lipolysis. Warrents et al. (1999) reported that when feeding tallow, linoleic acid incorporation into porcine adipose tissue was more rapid than its elimination. Omega-3 fatty acids are known to have anti-inflammatory effects, which may reduce basal lipolysis (Rustan et al., 1993; Calder, 2015). Rustan et al. (1993) reported that rats fed a diet with both fish oil and tallow as dietary fat sources versus just tallow decreased plasma NEFA levels and basal intracellular lipolysis by half.

Beta-oxidation

There are 2 major differences in the beta-oxidation pathway among fatty acids. The first is that short chain fatty acids (< C8:0) can passively diffuse the inner membrane of the mitochondria versus transporting it via the carnitine-transferase system (Odle et al., 1995). Thus, short chain fatty acids can be oxidized more rapidly than long chain fatty acids (Odle et al., 1995). The second difference is the amount of Acetyl-CoA, $FADH_2$, and NADH and resulting adenosine triphosphate (ATP) produced during the beta-oxidation process. For every 2 carbons in a fatty acid chain, 1 Acetyl-CoA, 1 $FADH_2$, and 1 NADH (exception for last 2 carbons of the fatty acid chain where just an Acetyl-CoA is produced) is produced via beta-oxidation (Jones and Rideout, 2012). However, if a double bond is present, the initial oxidation reaction via the Acyl-CoA dehydrogenase enzyme does not occur; thus the corresponding yield of 1 $FADH_2$ of that reaction does not occur (cost of ~2 ATP; Jones and Rideout, 2012). Thus, increased chain length

increases the total yield of ATP from beta-oxidation, while increased unsaturation of a fatty acid decreases ATP yield (Jones and Rideout, 2012).

Deposited fatty acid profile and carcass iodine value

For 90 years, it has been demonstrated and become accepted that the fatty acid composition of a dietary fat source will be highly reflected in the fatty acid composition of fat in the carcass (Ellis and Isbell, 1926). The chemical structure of a dietary fatty acid is largely unaltered from consumption to deposition (Allee et al., 1972). Studies have used serial slaughter and biopsies to determine changes in fatty acid composition during the finishing stage (Apple et al., 2009; Kellner et al., 2015). Based on these results, it has been determined that 50 to 60% of the change in the fatty acid composition of carcass or depot fat is due to changing the fat source or quantity in the diet (Apple et al., 2009). Therefore, it would seem highly probable that the lipid content of the diet could be used to predict the composition of carcass fat (Madsen et al., 1992; Boyd et al., 1997). The first attempt at such a prediction was reported over 50 years ago, resulting in the term iodine value product (IVP), a value that is based on an equation that includes both the dietary fat IV and the fat level in the diet times a constant of 0.10 (Christensen, 1962; Madsen et al., 1992). Recently, IVP has been increasingly used to predict carcass IV (Benz et al., 2011, Wu et. al, 2016). Flaws of IVP have been identified (Kellner et al., 2014), and it has been suggested that linoleic acid concentration or intake (Benz et al., 2011; Kellner et al., 2014) or additional factors such as energy intake would be more accurate in predicting carcass IV (Paulk et al., 2015; Wu et al., 2016). A list of equations predicting carcass iodine value (from backfat, belly fat, jowl fat, or a combination of the 3) from dietary fat and additional variables is presented in Table 1.7.

Estimated energy value

Dietary fat DE estimates have tended to increase as the unsaturated to SFA concentration ratio increases and the FFA level decreases (Powles et al., 1995; NRC, 2012), as previously explained. However, current predicative energy value equations based on the chemical composition of dietary fat sources have yet to be fully validated in commercial conditions (Boyd et al., 2015). It is currently theorized that any non-elutable material (i.e. moisture, volatile matter, insoluble material, impurities, and unsaponifiable matter) provides little energy to the diet; however, this has not yet been validated (Kerr et al., 2015).

Conversions of DE to ME and ME to NE are currently assumed to be the same across all compositions of dietary fat (NRC, 2012). However, as has been detailed in this review, it has been reported in multiple instances that feeding different dietary fat sources has resulted in changes in lipid metabolism, which in theory could result in substantial differences in the heat increment associated with a dietary fat source. Kil et al. (2011) reported no difference in the apparent total tract digestion of GE and acid hydrolyzed ether extract between choice white grease and soybean oil, even while there is 1.2 Mcal of NE/kg difference between the two sources.

Effects due to peroxidation of dietary fat

Process of dietary fat peroxidation

Unsaturated dietary lipid sources, animal protein meal, and cereal grain co-products such as distillers dried grains with solubles that are exposed to heat, as well as oxygen, light, moisture and heavy metals can all peroxidize (Belitz et al., 2009). Lipid peroxidation is a dynamic

process (Figure 1.5) that has been classified into 3 phases: initiation, propagation, and termination (Belitz et al., 2009). The initiation step produces free radicals which negativity affect lipid quality, while the propagation phase and termination phase produce ketones, aldehydes, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, which have a differing set of effects on lipid quality and resulting animal performance (Kerr and Shurson, 2012). To date, the complexity of products produced by peroxidation has resulted in a failure to find a single method that precisely predicts lipid composition and the resulting peroxidation impact on animal performance (Kim and LaBella, 1987; Kerr and Shurson, 2012).

Impacts of dietary fat peroxidation

Peroxidation of dietary lipids resulting in negative impacts on feed intake or growth has been found to be inconsistent (Kerr and Shurson, 2012). DeRouchey et al. (2004) reported that increasing the rancidity of choice white grease resulted in a decrease of feed intake, but digestibility of fatty acids was not impacted. Additionally, Fernandez-Duenas (2009) and Harrell et al. (2010) found that oxidized corn oil or distillers dried grains with solubles decreased growth performance. In contrast, Fernandez-Duenas et al. (2008) employed oxidized canola oil and tallow, and reported no effects on feed intake or growth. Clearly further understanding of the impact of individual peroxidation derived products on pig performance and health is needed. Antioxidants such as butylated hydroxytoluene, ethoxyquin, and tocopheral can be added to alleviate the negative effects of dietary oxidative stress (Frenandez-Duenas, 2009). Addition of antioxidants has resulted in mixed responses (Kerr and Shurson, 2012). The issue with antioxidant addition is that antioxidants cannot undo any peroxidation that has already taken place (Kerr et al., 2015). Data reported by Frenandez-Duenas (2009) and Harell et al. (2010)

show that antioxidant inclusion in oxidized corn oil and dried distillers grains with solubles improved growth performance, but Wang et al. (1997) and Song et al. (2013) reported no effects on growth performance through antioxidant inclusion.

Dietary fat analysis

Sampling

Few guidelines and instructions exist on how to properly sample a lipid source. Lipids by nature are hydrophobic. Thus, moisture and lipids within a source will naturally separate over time if contained in a static environment. Furthermore, FFA that are saturated are more hydrophobic than triglycerides and FFA that are poly-unsaturated (Liu et al., 2015). Thus, there is also a risk of segregating lipids based on chemical structure within a source. Consequently, it is important that when sampling a dietary fat source for analysis that multiple samples are taken from different locations within the source and that there is a thorough homogenization before analysis.

Fatty acid profile, chemical structure, and non-lipid content

Nutritionists, feed manufacturers, and scientists use a wide range of qualitative and quantitative methods to assess the quality of dietary fat sources (Table 1.8). Dietary fat sources can first be analyzed for percent tri-, di-, mono-glycerides, phospholipids, and FFA, as well as a fatty acid profile via gas chromatography to provide the molecular composition of the source of dietary fat (Kerr et al., 2015). These molecular analyses can be used to predict the energy value of the dietary fat source (Powles et al., 1995; Rosero et al., 2015) and the fatty acid composition of carcass fat (Kellner et al., 2014; Paulk et al., 2015). Furthermore, dietary fat sources can be

analyzed for acid value, iodine value, color, and their melting and solidification points; however, the impact of these generated values (if any) on the nutritional or energetic value of dietary fat source to the pig remains unclear (Kerr et al., 2015). Dietary fat sources can also be analyzed for non-lipid material such as non-elutable material (moisture, impurities, unsaponifiable material, glycerol, and oxidized/polymerized fats), metals (Ca, Cu, Fe, Mg, Mn, Na, P, and Zn), and antioxidant content.

Peroxidation

Dietary fat sources and lipids can also be analyzed to indicate the extent of or predict lipid peroxidation. As previously described, lipid peroxidation produces complex products which make analysis of lipid peroxidation difficult (Kim and LaBella, 1987; AOCS, 2005). A description of assays used to determine the extent of lipid peroxidation are listed in Table 1.9. The most widely used tests are peroxide value, anisidine value and thiobarbituric acid reactive substances (TBARS; NRC, 2012). Peroxide value quantifies products of peroxidation generated in the initiation phase, while anisidine value and TBARS quantify products generated in the propagation phase (Ross and Smith, 2006). However, these analyses do not measure compounds that remained unchanged during the process of peroxidation, nor do they capture the hydroperoxides and aldehydes that have been degraded as peroxidation continues (Kerr et al., 2015). Thus, there is a clear need for new and more reliable methods using liquid or gas chromatography to quantify the extent of lipid peroxidation (NRC, 2012; Kerr et al., 2015). The most common methods to quantify the remaining lipid peroxidation potential are the active oxygen method and the oil stability index (Shahidi and Wanasundara, 1996). To date, there is no single index or combination of indices that provides an accurate way to predict the impact the

peroxidation of a dietary fat source has on the pig's response to dietary fat (Kerr and Shurson, 2012).

Diet, ileal, and fecal lipid analysis

The use of a similar analyses across experiments and laboratories for lipid determination is necessary for an unbiased understanding of digestion across differing scenarios (NRC, 2012). Currently the lipid content of diet, intestinal digesta, or fecal matter is determined through multiple procedures (Hammond, 2001; NRC, 2012). Variances of these procedures include solvent type (ether, hexanes, or chloroform), extraction time, temperature, pressures, and sample dryness (Matthaus and Bruhl, 2001; NRC, 2012). Crude fat extraction methods do not completely extract fatty acids, especially if they are linked to carbohydrates or proteins, or present as salts of divalent cations (Palmquist and Jenkins, 2003). Extraction of lipids using initial acid-hydrolysis corrects for this by breaking fatty acids away from: tri- di- and mono-glycerides, lipid-carbohydrate bonds, lipid-protein bonds, sterols, and phospholipids, resulting in a more thorough extraction (Palmquist and Jenkins, 2003). Therefore, concentrations of lipids are usually higher when acid-hydrolysis is employed prior to extraction (Palmquist and Jenkins, 2003; NRC, 2012). Due to the potential presence of cation-bound lipids in collected ileal material, it is strongly suggested that all lipid analysis be performed using the same procedures (NRC, 2012). New technologies such as liquid chromatography, nuclear magnetic resonance, and near-infrared spectroscopy are more rapid, but to date their true value is uncertain. (NRC, 2012).

Additional positive attributes of dietary fat inclusion in swine diets

Heat stress

Use of fat in commercial swine diets in North America is typically greatest during the warm summer months. Heat stress affects a plethora of swine production variables (Baumgard et al., 2012); its negative impact on average daily gain in pigs has been known for over 110 years (Heitman et al., 1958). Despite improvements in barn design, genetics, management, and nutrition, heat stress remains one of the most costly issues for U.S. pork producers (St-Pierre et al., 2003; Renaudeau et al., 2012). To reduce the negative impact of heat stress on energy intake (Hao et al., 2014; Pearce et al., 2014), producers formulate diets using ingredients that are energy dense and low in heat increment (Forbes and Swift, 1944; Stahly et al., 1981). Adding dietary fat has been shown to reduce but by no means completely mitigate the negative effects of HS on ADG (Stahly et al., 1981; Spencer et al., 2005).

It is unclear if dietary fat is utilized similarly between thermoneutral conditions and during bouts of heat stress. A review by Baumgard and Rhoads (2013) concluded that pigs that experience heat stress deposit more lipid than their energy consumption predicted. The retention of stored triglycerides in adipose tissue during heat stress when energy intake is decreased is the opposite of what occurs during thermoneutral conditions when energy intake is decreased. Under thermoneutral conditions, there is a classic catabolic response where stored lipids are mobilized, while circulating NEFA concentrations and whole body oxidation are increased (Vernon, 1992). Reduced lipolysis in adipose tissue may be an attempt to reduce thermogenesis during mitochondrial fatty acid transport and β-oxidation (Mujahid and Furuse, 2008). Another potential explanation is that insulin, an acute anabolic and anti-lipolytic hormone, is increased in circulating concentration during heat stress (Baumgard and Rhoads, 2013). Thus, while adding

dietary fat during the summer months increases dietary energy concentration with the added bonus of lowering the heat increment, it is a potential concern that during heat stress the additional dietary fat is being mostly used for fat deposition and is not available as an energy source for other tissues and processes.

Manufacturing

Dietary fat inclusion can reduce dust associated with feed manufacturing and handling, and improve pellet manufacturing and quality (Carter, 2010). However, depending on the feed handling system, a dietary fat inclusion of > 5% will increase the risk of feed flow issues (Carter, 2010; NRC, 2012).

Conclusion

The primary purpose of dietary fat in swine diets is to provide energy. A better understanding of how a chemically diverse range of dietary fat sources (Figure 1.6) are digested, absorbed, circulated, deposited, and metabolized by the growing pig will create a more accurate and precise energy value. Such research will allow pork producers to include dietary fat with greater confidence and profitability. This review indicates that data are needed: to validate current DE, ME and NE estimates of dietary fat; to determine if dietary fat is utilized by the pig similarly during thermoneutral and heat stress conditions; and to further quantify the effects of the chemical composition of dietary fat on digestion and metabolism of lipids in the growing pig.

Literature cited

AAFCO. 2011. Official Publication of the Association of American Feed Control Officials. Assoc. Am. Feed Control Off., Washington, DC.

Ackrell, B. A. 1974. Metabolic regulatory functions of oxaloacetate. Horiz. Biochem Biophys. 1:175-219.

Acosta, J. A., R. D. Boyd, and J. F. Patience. 2015. Endogenous and exogenous fat digestion in growing pigs. J. Anim. Sci. 93(Suppl 2):75.

Adams, K. L., and A. H. Jensen. 1984. Comparative utilization of in-seed fats and the respective extracted fats by the young pig. J. Anim. Sci. 59:1557-1566. doi:10.2527/jas1984.5961557x.

Adams, K. L., and A. H. Jensen. 1985. Effect of processing on the utilization by young pigs of the fat in soya bean and sunflower seeds. Anim. Sci. Feed Tech. 12:267-274. doi.org/10.1016/0377-8401(85)90003-3.

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern editors, Swine Nutrition, 2nd ed. CRC Press, Boca Raton, Florida. p. 903-916.

Adeola, O., D. C. Mahan, M. J. Azain, S. K. Baidoo, G. L. Cromwell, G. M. Hill, J. E. Pettigrew, C. V. Maxwell, and M. C. Shannon. Dietary lipid sources and levels for weanling pigs. J. Anim. Sci. 91:4216-4225. doi:10.2527/jas.2013-6297.

Akram, M. 2013. A focused review of the role of ketone bodies in health and disease. J. Med. Food 16:965-967. doi:10.1089/jmf.2012.2592.

Allee, G. L., D. H. Baker, and G. A. Leveille. 1971. Influence of level of dietary fat on adipose tissue lipogenesis and enzymatic activity in the pig. J. Anim. Sci. 33:1248-1254. doi:10.2527/jas1971.3361248x.

Allee, G. L., D. R. Romsos, G. A. Leveille and D. H. Baker. 1972. Lipogenesis and enzymatic activity in pig adipose tissue as influenced by source of dietary fat. J. Anim. Sci. 35:41-47. doi:10.2134/jas1972.35141x.

AOCS. 2005. Analysis of lipid oxidation. In: A. Kamal-Eldin and J. Koromy editors, AOCS Press, Champaign, IL.

Apple, J. K., C. V. Maxwell, D. L. Galloway, S. Hutchison, and C. R. Hamilton. 2009. Interactive effects of dietary fat source and slaughter weight in growing-finishing swine: II. Fatty acid composition of subcutaneous fat. J. Anim. Sci. 87:1423–1440. doi:10.2527/jas.2008-1455.

Atwater, W. O. 1900. Discussion of the terms digestibility, availability, and fuel value. In: 12th Annual report of Storrs Agr. Exp. Sta. p 69-110.

Averette Gatlin, L. A., M. T. See, and J. Odle. 2005. Effects of chemical hydrogenation of supplemental fat on relative apparent lipid digestibility in finishing swine. J. Anim. Sci. 83:1890-1898. doi:10.2527/2005.8381890x.

Bach, A. C., and V. K. Babayan. 1982. Medium-chain triglycerides: an update. Am. J. Clin. Nutr. 36:950-962.

Bartelett, J. M. S. and D. Stirling. 2003. A short history of the polymerase chain reaction. In: J. M. S. Bartlett and D. Stirling editors, PCR Protocols. Human Press, Totowa, NJ. p. 3-7.

Baumgard, L. H., R. P. Rhoads, M. L. Rhoads, N. K. Gabler, J. W. Ross, A. F. Keating, R. L. Boddicker, S. Lenka, and V. Sejian. 2012. Impact of climate change on livestock production. In: V. Sejian, S. M. K. Naqvi, T. Ezeji, J. Lakritz, and R. Lal, editors, Environmental stress and amelioration in livestock production. Springer-Verlag, Berlin, Germany. p. 413–468.

Baumgard, L. H., and R. P. Rhoads. 2013. Effects of heat stress on post-absorptive metabolism and energetics. Annu. Rev. Anim. Biosci. 1:311–337.

Bayley, H. S., and D. Lewis. 1965. The use of fats in pig feeding. I. Pig faecal fat not of immediate dietary origin. J. Agric. Sci. Camb. 64:367-372.

Bee, G., S. Gebert, and R. Messikommer. 2002. Effect of dietary energy supply and fat source on the fatty acid pattern of adipose and lean tissues and lipogenesis in the pig. J. Anim. Sci. 80:1564–1574. doi:10.2527/2002.8061564x.

Bee, G., S. Jacot, G. Guex, and C. Biolley. 2008. Effects of two supplementation levels of linseed combined with CLA or tallow on meat quality traits and fatty acid profile of adipose and different muscle tissues in slaughter pigs. Animal. 2:800-811. doi:10.1017/S175173110800181X.

Beld, J., D. J. Lee, and M. D. Burkart. 2015. Fatty acid biosynthesis revisited: structure elucidation and metabolic engineering. Mol. Biosyst. 11:38-59. doi:10.1039/c4mb00443d.

Belitz, H. D., W. Grosch, and P. Schieberle. 2009. Lipids. In: H. D. Belitz, W. Grosch, and P. Schieberle, editors, Food chemistry. Springer, Berlin, Germany.

Benz, J. M., M. D. Tokach, S. S. Dritz, J. L. Nelssen, J. M. DeRouchey, R. C. Sulabo, and R. D. Goodband. 2011. Effects of dietary iodine value product on growth performance and carcass fat quality of finishing pigs. J. Anim. Sci. 89:1419–1428. doi:10.2527/jas.2010-3126.

Bergstrom, S., and B. Borgstrom. 1956. Metabolism of lipids. Annu. Rev. Biochem. 25:177-200.

Birk, R. Z., K. S. Regan, E. Boyle-Roden, and P. M. Brannon. 2004. Pancreatic lipase and its related protein 2 are regulated by dietary polyunsaturated fat during the postnatal period development in rats. Pediatr. Res. 56:256-262.

Boisen, S., and J. A. Fernandez. 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim. Feed Sci. Technol. 68:277-286. doi.org/10.1016/S0377-8401(97)00058-8.

Borgstrom, B. 1980. Importance of phospholipids, pancreatic phospholipase A2, and fatty acid for the digestion of dietary fat: in vivo experiments with porcine enzymes. Gastroenterology. 78:954-962.

Borgstrom, B., and C. Erlanson. 1973. Pancreatic lipase and colipase interactions and effects of bile salts and other detergents. Eur. J. Biochem. 37:60-68.

Boyd, R. D., C. E. Zier-Rush, M. McGrath, R. Palan, J. Picou, and E. van Heugten. 2015. Calibration of net energy for fat by growth assay in early and late phases of growth in pigs. J. Anim. Sci. 93(Suppl 2):73.

Boyd, R. D., M. E. Johnston, K. Scheller, A. A. Sosnicki, and E. R. Wilson. 1997. Relationship between dietary fatty acid profile and body fat composition in growing pigs. PIC Technical Memo 153. PIC, Franklin, KY.

Bracco, U. 1994. Effect of triglyceride structure on fat absorption. Am. J. Clin. Nutr. 60:1002S-1009S.

Calder, P. C. 2015. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta. 2015 1851:469-484. doi:10.1016/j.bbalip.2014.08.010.

Carter, S. D. 2010. Energy sources for swine diets. In: National swine nutrition guide. p. 22-29.

Cera, K. R, D. C. Mahan, and G. A. Reinhart. 1988. Weekly digestibilities of diets supplemented with corn oil, lard, or tallow by weanling swine. J. Anim. Sci. 66:1430-1437. doi:10.2134/jas1988.6661430x.

Cera. K. R., D. C. Mahan, and G. A. Reinhart. 1989. Apparent fat digestibilities and performance responses of postweaning swine fed diets supplemented with coconut oil, corn oil or tallow. J. Anim. Sci. 67:2040-2047. doi:10.2527/jas1989.6782040x.

Cera, K. R., D. C. Mahan, and G. A. Reinhart. 1990. Evaluation of various extracted vegetable oils, roasted soybeans, medium chain triglyceride and an animal-vegetable fat blend for postweaning swine. J. Anim. Sci. 68:2756-2765. doi:10.2527/1990.6892756x

Chow, S. L., and D. Hollander. 1979. A dual, concentration-dependent absorption mechanism of linoleic acid by rat jejunum in vitro. J. Lipid. Res. 20:349-356.

Christensen, K. D. 1962. Foderfedtets indflydelse pa smorrets og flaeskets kvalitet. Thesis, Kgl. Vet.- og Landbohojsk., Kobenhavn, p. 88 (Danish).

Clarke, S. D. 1993. Regulation of fatty acid synthase gene expression: an approach for reducing fat accumulation. J. Anim. Sci. 71:1957-1965. doi:10.2527/1993.7171957x.

Cromwell, G. L., M. J. Azain, O. Adeola, S. K. Baidoo, S. D. Carter, T. D. Crenshaw, S. W. Kim, D. C. Mahan, P. S. Miller, and M. C. Shannon. 2011. Corn distillers dried grains with solubles in diets for growing-finishing pigs: A cooperative study. J. Anim. Sci. 89:2801-2811. doi:10.2527/jas.2010-3704.

Cunningham, H. M., and W. M. F. Leat. 1969. Lipid synthesis by the monoglyceride and α-glycerophosphpate pathways in sheep intestine. Can. J. Biochem. 47:1013-1020.

Danis, P., and R. Farkas. 2009. Hormone-dependent and hormone-independent control of metabolic and developmental functions of malate dehydrogenase. Endocr. Regul. 43:39-52.

Denton, R. M., P. J. Randle, B. J. Bridges, R. H. Cooper, A. L. Kerbey, H. T. Pask, D. L. Severson, D. Stansbie, and S. Whitehouse. 1975. Regulation of mammalian pyruvate dehydrogenase. Mol. Cell Biochem. 31:27-53.

DeRouchey, J. M., J. D. Hancock, R. D. Hines, C. A. Maloney, D. J. Lee, H. Cao, D. W. Dean, and J. S. Park. 2004. Effects of rancidity and free fatty acids in choice white grease on growth performance and nutrient digestibility in weanling pigs. J. Anim. Sci. 82:2937-2944. doi:10.2527/2004.82102937x.

Di Luca, A., G. Elia, R. Hamill, and A. M. Mullen. 2013. 2D DIGE proteomic analysis of early post mortem muscle exudate highlights the importance of the stress response for improved water-holding capacity of fresh pork meat. Proteomics. 13:1528-44. doi:10.1002/pmic.201200145.

Duran-Montge, P., R. Lizardo, D. Torrallardona, and E. Esteve-Garcia. 2007. Fat and fatty acid digestibility of different fat sources in growing pigs. Livest. Sci. 109:66-69. doi.org/10.1016/j.livsci.2007.01.067.

Ellis, N. R., and H. S. Isbell. 1926. Soft Pork Studies. II. The influence of the character of the ration upon the composition of the body fat of hogs. J. Biol. Chem. 59:219-248.

Enser, M. 1984. The chemistry, biochemistry and nutritional importance of animal fats. In: J. Wiseman, editor, Fats in animal nutrition. Butterworths, London, UK. P. 23-51.

Estrada Restrepo, J. E. 2013. Factors associated with variation in the fatty acid composition and iodine value of carcass fat in pigs fed increasing levels of dried distillers grains with solubles. M.S. Thesis. Univ. of Illinois at Urbana-Champaign, Champaign.

Eusebio, J. A., V. W. Hays, V. C. Speer, and J. T. Mccall. 1965. Utilization of fat by young pigs. J. Anim. Sci. 24:1001-1007. doi:10.2527/jas1965.2441001x.

Fernandez-Duenas, D. M. 2009. Impact of oxidized corn oil and synthetic antioxidant on swine performance, antioxidant status of tissues, pork quality and shelf life evaluation. Ph. D. dissertation thesis, Urbana, IL.

Flatt, J. P. 1970. Conversion of carbohydrate to fat in adipose tissue: an energy-yielding, and, therefore, self-limiting process. J. Lipid Res. 11:131-143.

Forbes, E. B., and R. W. Swift. 1944. Associative dynamic effects of protein, carbohydrate and fat. Science. 99:476-478. doi:10.1126/science.99.2580.476.

Frobish, L. T., and V. W. Hays, V. C. Speer, and R. C. Ewan. 1970. Effect of fat source and level on utilization of fat by young pigs. J. Anim. Sci. 30:197-202. doi:10.2527/jas1970.302197x.

Galloway, S. T., and R. C. Ewan. 1989. Energy evaluation of tallow and oat groats for young swine. J. Anim. Sci. 67:1744-1750. doi:10.2527/jas1989.6771744x.

Geng, Y. Y., F. H. Yang, X. M. Xing, and X. H. Gao. 2012. Effects of dietary fat levels on nutrient digestibility and production performance of growing-furring blue foxes (Alopex lagopus). J. Anim. Physiol. Anim. Nutr. (Berl). 96:610-617. doi:10.1111/j.1439-0396.2011.01186.x.

Goncharova, K., S. G. Pierzynowski, D. Grujic, and S. Kirko. 2014. A piglet with surgically induced exocrine pancreatic insufficiency as an animal model of newborns to study fat digestion. Br. J. Nutr. 112:2060-2067. doi:10.1017/S0007114514003286.

Greenbaum, D., C. Colangelo, K. Williams, and M. Gerstein. 2003. Comparing protein abundance and mRNA expression levels on a genomic scale. Genome Bio. 4:117-130. doi:10.1186/gb-2003-4-9-117.

Gutierrez, N. A., N. V. L. Serao, and J. F. Patience. 2016. Effects of distillers' dried grains with solubles and soybean oil on dietary lipid, fiber, and amino acid digestibility in corn-based diets fed to growing pigs. J. Anim. Sci. 94:1508-1519. doi:10.2527/jas.2015-9529.

Hamilton, R. M., and B. E, McDonald. 1969. Effect of dietary fat source on the apparent digestibility of fat and the composition of fecal lipids of the young pig. J. Nutr. 97:33-41.

Hammond, E. W. 2001. Lipid analysis – a 20th century success? J. Sci. Food Agri. 82:5-11. doi:10.1002/jsfa.1031.

Hamosh, M. 1990. Lingual and gastric lipases. Nutrition. 6: 421-428.

Harrell, R. J., J. Zacho, G. Reznik, D. Macaraeg, T. Wineman, and J. Richards. 2010. Application of a blend of dietary antioxidants in nursery pigs fed either fresh or oxidized corn oil of DDGS. J. Anim. Sci. 88(Suppl. 3):97.

Harwood, J. L., and C. M. Scrimgeour. 2007. Fatty acid and lipid structure. In: The lipid handbook. 3rd ed. CRC Press. Boca Raton, FL. p. 1-36.

Heckler, B. K. 1997. Lactate production by swine adipocytes: effects of age, nutritional status, glucose concentration, and insulin. Am. J. Physiol. 272:957-966.

Heitman, H., C. F. Kelly, and T. E. Bond. 1958. Ambient air temperature and weight gain in swine. J. Anim. Sci. 17:62-67. doi:10.2134/jas1958.17162x.

Hennessy, A. A., P. R. Ross, G. F. Fitzgerald, and C. Stanton. 2016. Sources and bioactive properties of conjugated dietary fatty acids. Lipids. 51:377-397. doi:10.1007/s11745-016-4135-z.

Hoffmann, A. F., and H. F. Mekhijian. 1973. Bile acids and the intestinal absorption of fat and electrolytes in health and disease. In: P. P. Nair, D. Kritchevsky, editors, The bile acids, vol 2., New York, NY. Plenum Press.

Jenkins, C. M., D. J. Mancuso, W. Yan, H. F. Sims, B. Gibson, and R. W. Gross. 2004. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. J. Biol. Chem. 279:48698-48975. doi:10.1074/jbc.M407841200.

Johnston, J. M., and B. Borgstrom. 1964. The intestinal absorption and metabolism of micellar solution on lipids. Biochem. Biophys. Acta. 84:412-423.

Jones, D. B., J. D. Hancock, D. L. Harmon, and C. E. Walker. 1992. Effects of exogenous emulsifiers and fat sources on nutrient digestibility, serum lipids, and growth performance in weanling pigs. J. Anim. Sci. 70:3473-3482. doi:10.2527/1992.70113473x.

Jones, P. J. H., and T. Rideout. 2012. Lipids, sterols, and their metabolites. In: A. C. Ross, B. Caballero, R. J. Cousins, K. L. Tucker, and T. R. Ziegler, editors, Modern nutrition in health and disease. 11th rev. ed. Lippincott Williams and Wilkins. Philadelphia, PA. p. 65-87.

Jorgensen, H., and J. A. Fernandez. 2000. Chemical composition and energy value of different fat sources for growing pigs. Acta Agric. Scand. A Anim. Sci. 50:129-136.

Jorgensen, H., V. M. Gabert, M. S. Hedemann, and S. K. Jensen. 2000. Digestion of fat does not differ in growing pigs fed diets containing fish oil, rapeseed oil, or coconut oil. J. Nutr. 130:852-857.

Jorgensen, H., S. K. Jensen, and B. O. Eggum. 1996. The influence of rapeseed oil on digestibility, energy metabolism and tissue fatty acid composition in pigs. Acta. Agri. Scand. A. Anim. Sci. 46:65-75.

Jorgensen, H., K. Jakobsen, and B. O. Eggum. 1993. Determination of endogenous fat and fatty acids at the terminal ileum and on faeces in growing pigs. Acta Agric. Scand. A Anim. Sci. 43:101-106.

Jorgensen, H., K. Jakobsen, and B. O. Eggum. 1992. The influence of different protein, fat and mineral levels on the digestibility of fat and fatty acids measured at the terminal ileum and in feces of growing pigs. Acta. Agric. Scand. Sect. A Anim. Sci. 43:101-106.

Kellner, T. A., K. J. Prusa, and J. F. Patience. 2014. Impact of dietary fat source and concentration and daily fatty acid intake on the composition of carcass fat and iodine value sampled in three regions of the pork carcass. J. Anim. Sci. 92:5485-5495 doi:10.2527/jas.2014-7567.

Kerr, B. J., T. A. Kellner, and G. C. Shurson. 2015. Characteristics of lipids and their feeding value in swine diets. J. Anim. Sci. Biotechnol. 6:30. doi:10.1186/s40104-015-0028-x.

Kerr, B. J., and G. C. Shurson. 2012. Lipids, fatty acids, and more. Midwest Swine Nutrition Conference Proceedings. p. 25-36.

Kerr, B. J., T. E. Weber, W. A. Dozier, III, and M. T. Kidd. 2009. Digestible and metabolizable energy content of crude glycerin originating from difference sources in nursery pigs. J. Anim. Sci. 87:4042-4049. doi:10.2527/jas.2008-1676.

Kil, D. Y., F. Ji, L. L. Stewart, R. B. Hinson, A. D. Beaulieu, G. L. Allee, J. F. Patience, J. E. Pettigrew, and H. H. Stein. 2011. Net energy of soybean oil and choice white grease in diets fed to growing and finishing pigs. J. Anim. Sci. 89:448-459. doi:10.2527/jas.2010-3233.

Kil, D. Y., T. E. Sauber, D. B. Jones, and H. H. Stein. 2010. Effect of the form of dietary fat and the concentration of dietary neutral detergent fiber of ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. J. Anim. Sci. 88:2959-2967. doi:10.2527/jas.2009-2216.

Kim, B. G., D. Y. Kil, and H. H. Stein. 2013. In growing pigs, the true and total tract digestibility of acid hydrolyzed ether extract in extracted corn oil is greater than in intact sources of corn oil or soybean oil. J. Anim. Sci. 91:755-763. doi:10.2527/jas.2011-4777.

Kim, R. S., and F. S. LaBella. 1987. Comparison of analytical methods for monitoring autoxidation profiles of authentic lipids. J. Lipid Res. 28:1110-1117.

Kindel, T., D. M. Lee, and P. Tso. 2010. The mechanism of the formation and secretion of chylomicrons. Atheroscler. Suppl. 11:11-16.

Kloareg, M., J. Noblet, and J. van Milgen. 2007. Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. Br. J. Nutr. 97:35–44. doi:10.1017/S0007114507205793.

Klose, J. 1975. Protein mapping by combined isoelectric focusing and electrophoresis of mouse tissues. A novel approach to testing for induced point mutations in mammals. Humangenetik. 26:231-243.

Koch, D. E., A. M. Pearson, W. T. Magee, J. A. Hoefer, and B. S. Schweigert. 1968. Effect of diet on the fatty acid composition of pork fat. J. Anim. Sci. 27:360-365. doi:10.2527/jas1968.272360x.

Kouba, M., M. Bonneau, and J. Noblet. 1999. Relative development of subcutaneous, intermuscular, and kidney fat in growing pigs with different body compositions. J. Anim. Sci. 77:622-629. doi:/1999.773622x.

Lane, M. D., J. Moss, and S. E. Polakis. 1974. Acetyl coenzyme A carboxylase. Curr. Top. Cell Regul. 8:139-195.

Langlois, A., T. Corring, F. Levenez, J. C. Cuber, and J. A. Chayvialle. 1990. Effects of pancreatic polypeptide on biliary flow and bile acid secretion stimulated by secretin and cholecystokinin in the conscious pig. Regul. Pept. 27:139-147.

Lawes, J. F., and J. H. Gilbert. 1886. On the source of fat of the animal body. Phil. Mag. 4:439-451.

Lemarie F., J. F. Cavalier, C. Garcia, F. Biossel, V. Point, D. Catheline, P. Legrand, F. Carriere, and V. Rioux. 2016. Effect of preduodenal lipase inhibition in suckling rats on dietary octanoic acid (C8:0) gastric absorption and plasma octanoylated ghrelin concentration. Biochim Biophys Acta. 1861:1111-1120. doi:10.1016/j.bbalip.2016.06.009.

Liu, M. S., P. Green, J. J. Mann, S. I. Rapoport, and M. E. Sublette. 2015. Pathways of polyunsaturated fatty acid utilization: implications for brain function in neuropsychiatric health and disease. Brain. Res. 9:220-246. doi:10.1016/j.brainres.2014.11.059.

Lizardo, R., J. van Milgen, J. Mourot, J. Noblet, and M. Bonneau. 2002. A nutritional model of fatty acid composition in the growing-finishing pig. Livest. Prod. Sci. 75:167-182.

Lloyd, L. E., E. W. Crampton, and V. G. MacKay. 1957. The digestibility of ration nutrients by three vs. seven week old pigs. J. Anim. Sci. 16:383-388. doi:10.2527/jas1957.162383x.

Madsen, A., K. Jakobsen, and H. Mortensen. 1992. Influence of dietary fat on carcass fat quality in pigs. A review. Acta. Agric. Scand. 42:220-225.

Mahmmod, T., and P. C. Yang. 2012. Western blot: technique, theory, and trouble shooting. North. Am. J. Med. Sci. 4:429-434. doi:10.4103/1947-2714.100998.

Matthaus, B., and L. Bruhl. 2001. Comparison of different methods for the determinations of the oil content in oilseeds. Jour. A.O.C.S. 78:95-102.

Maynard, L. A. 1944. The Atwater system of calculating the caloric value of diets. J. Nutr. 74:443-452.

Mears, G. J., and V. E. Mendel. 1974. Glucose metabolism and lipid mobilization by adipocytes from mice selected for growth. J. Physiol. 240:609-624.

Mendoza, S. M., and E. van Heugten. 2014. Effects of dietary lipid sources on performance and apparent total tract digestibility of lipids and energy when fed to nursery pigs. J. Anim. Sci. 92:627-636. doi:10.2527/jas2013-6488.

Minich, D. M., R. J. Vonk, and H. J. Verkade. 1997. Intestinal absorption of essential fatty acids under physiological and essential fatty acid-deficient conditions. J. Lipid Res. 38:1709-1721.

Mourot, J., P. Peiniau, and A. Mounier. 1994. Effect of dietary linoleic-acid on lipogenesis in adipose-tissue of pig. Reprod. Nutr. Dev. 34:213–220.

Mujahid, A., and M. Furuse. 2008. Central administration of corticotropin-releasing factor induces thermogenesis by changes in mitochondrial bioenergetics in neonatal chicks. Neuroscience. 155:845-851. doi:10.1016/j.neuroscience.2008.06.026.

Nawar, W. W. 1996. Lipids. In: O. R. Fennema editor, Food Chemistry, 3rd ed. Marcel Dekker, Inc. New York, New York. p. 225-319.

Noblet, J., H. Fortune, C. Dupire, and S. Dubois. 1993. Digestible, metabolizable and net energy values of 13 feedstuffs for growing pigs: Effect of energy system. Anim. Feed Sci. and Tech. 42:131-149.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

O'Brein, D. R. 2009. Fats and oils: formulating and processing for application. 3rd ed. Boca Raton, FL: CRC Press.

Odle, J. 1997. New insights into the utilization of medium-chain triglycerides by the neonate: observations from a piglet model. J. Nutr. 127:1061–1067.

O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250:4007-4021.

O'Hea, E. K., and G. A. Leveille. 1969. Significance of adipose tissue and liver as sites of fatty acid synthesis in the pig and the efficiency of utilization of various substrates for lipogenesis. J. Nutr. 99:338-344.

Palmquist, D. L., and Jenkins T. C. 2003. Challenges with fats and fatty acid methods. J. Anim. Sci. 81:3250-3254. doi:10.2527/2003.81123250x.

Patience, J. F. 2012. The influence of dietary energy on feed efficiency in grow-finish swine. In: J. F. Patience, editor, Feed efficiency in swine. Wageningen Academic Publishers, Wageningen, the Netherlands. p. 101-129.

Paulk, C. B., J. R. Bergstrom, M. D. Tokach, S. S. Dritz, D. D. Burnett, E. W. Stephenson, M. A. Vaughn, J. M. DeRouchey, R. D. Goodband, J. L. Nelssen, and J. M. Gonzalez. 2015. Equations generated to predict iodine value of pork carcass back, belly, and jowl fat. J. Anim. Sci. 93:1666–1678. doi:10.2527/jas2014-8400.

Pearce, S. C., S. M. Lonergan, E. Huff-Lonergan, L. H. Baumgard, and N. K, Gabler. 2015. Acute heat stress and reduced nutrient intake alter intestinal proteomic profile and gene expression in pigs. PLoS ONE 10:e0143099. doi:10.1371/journal.pone.0143099.

Pearce, S. C., M. V. Sanz Fernandez, J. H. Hollis, L. H. Baumgard, and N. K. Gabler. 2014. Short-term exposure to heat stress attenuates appetite and intestinal integrity in growing pigs. J. Anim. Sci. 92:5444-5454. doi:10.2527/jas2014-8407.

Peirson, S. N., and J. N. Butler. 2007. Quantitative polymerase chain reaction. Methods Mol. Biol. 362:349-362.

Powles, J., J. Wiseman, D. J. A. Cole, and S. Jagger. 1995. Prediction of the apparent digestible energy value of fats given to pigs. Anim. Sci. 61:149-154. doi:1357-7298/95/45810149S20.00111.

Powles, J. J. Wiseman, D. J. A. Cole, and B. Hardy. 1994. Effect of chemical structure of fats upon their apparent digestible energy value when given to growing/finishing pigs. Anim. Prod. 57:137-146.

Powles, J., J. Wiseman, D. J. A. Cole, and B. Hardy. 1993. Effect of Effect of chemical structure of fats upon their apparent digestible energy value when given to growing/finishing pigs. Anim. Prod. 57:137-146.

Raclot, T., and R. Groscolas. 1995. Selective mobilization of adipose tissue fatty acids during energy depletion in the rat. J. Lipid Res. 36:2164–2173.

Remington, S. J. 1992. Structure and mechanism of citrate synthase. Curr. Top. Cell. Regul. 33:209-229.

Renaudeau, D., H. Gilbert, and J. Noblet. 2012. Effect of climatic environment on feed efficiency in swine. In: J. F. Patience, editor, Feed efficiency in swine. Wageningen Academic Publishers, Wageningen, the Netherlands. p. 183-210.

Rosero, D. S., J. Odle, C. Arellano, R. D. Boyd, and E. van Heugten. 2015. Development of prediction equations to estimate the apparent digestible energy content of lipids when fed to lactating sows. 93:1165-1176. doi:10.2527/jas.2014-8402.

Ross, C. F., and D. M. Smith. 2006. Use of volatiles as indicators of lipid oxidation in muscle foods. Comp. Rev. Food Sci. and Food Safety. 5:18-25. doi:10.1111/j.1541-4337.2006.tb00077.x.

Rustan, A. C., B. E. Hustvedt, and C. A. Drevon. 1993. Dietary supplementation of very long-chain n-3 fatty acids decreases whole body lipid utilization in the rat. J. Lipid Res. 34:1299-1309.

Sabesin, S. M., and S. Frase. 1977. Electron microscopic studies of the assembly, intracellular transport and secretion of chylomicrons by rat intestine. J. Lipid. Res. 18:496-511.

Saunders, W. B., and Ginsberg, H. N. 1994. Lipoprotein metabolism and its relationship to atherosclerosis. Med. Clin. North Am. 78:1-20.

Sauvant, D., J. M. Perex, and G. Tran. 2004. Tables of composition and nutritional value of feed materials, INRA, Paris, France, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.

Scopes, R. K. 2002. Enzyme activity and assays. p. 1-6. doi:10.1038/npg.els.0000712.

Scrimgeour, C. 2005. Chemistry of fatty acids. In: S. Fereidoon, editor. Bailey's Industrial Oil and Fat Products 6th ed. p. 1-43.

Scrimgeour, C., and J. L. Hardwood. 2007. Fatty acid and lipid structure. In: F. D. Gunstone, J. L. Hardwood, and A. J. Dijkstra, editors. The lipid handbook. 3rd ed. p. 1-36.

Shahidi, R., and J. N. Wanasundara. 1996. Methods for evaluation of the oxidative stability of lipid-containing foods. Food Sci. Technol. Int. 2:73-81.

Shiau, Y. F. 1981. Mechanisms of intestinal fat absorption. Am. J. Physiol. 240:G1-9.

Smith, D. R., D. A. Knabe, and S. B. Smith. 1996. Depression of lipogenesis in swine adipose tissue by specific dietary fatty acids. J. Anim. Sci. 74:975-983. doi:10.2527/1996.745975x.

Song, R., C. Chen, L. Wang, L. J. Johnson, B. J. Kerr, T. E. Weber, and G. C. Shurson. 2013. High sulfur content in corn dried distillers grains with solubles protects against oxidized lipids by increasing sulfur-containing antioxidants in nursery pigs. 91:2715-2728.

St-Pierre, N., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. J. Dairy Sci. 86:E52–E77.

Spencer, J. D., A. M. Gaines, E. P. Berg, and G. L. Allee. 2005. Diet modifications to improve finishing pig performance and pork quality attributes during periods of heat stress. J. Anim. Sci. 83:243-254. doi:/2005.831243x.

Stahly, T. S. 1984. Use of fats in diets for growing pigs. In: J. Wiseman, editor, Fats in animal nutrition. Butterworths. London, England. p. 313-331.

Stein, H. H., B. Seve, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172-180. doi:10.2527/jas.2005-742.

Stremmel, W., L. Pohl, A. Ring, and T. Hermann. 2001. A new concept of cellular uptake and intracellular trafficking of long-chain fatty acids. Lipids. 36:981-989.

Tancharoenrat, P., V. Ravindran, F. Zaefarian, and G. Ravindran. 2014. Digestion of fat and fatty acids along the gastrointestinal tract of broiler chickens. Poult. Sci. 93:371-379. doi.org/10.3382/ps.2013-03344.

Tichopad, A., M. Dilger, G. Schawrz, and M. W. Pfaffl. 2003. Standardized determination of real-time PCR efficiency from a single reaction set-up. Nucleic. Acids. Res. 31:6688.

Tou, J. C., S. N. Altman, J. C. Gigliotti, V. A. Benedito, and E. L. Cordonier. 2011. Different sources of omega-3 polyunsaturated fatty acids affects apparent digestibility, tissue deposition, and tissue oxidative stability in growing female rats. Lipids Health Dis. 10:179.

Towbin, M. 1979. Western blotting: Electrophoretic transfer of proteins from sodium dodecyl sulphate--polyacrilamide gels to unmodified nitrocellulose and radiographic detection with antibodies and radio-iodinated protein. Proc. Natl. Acad. Sci. U.S.A. 76:43.

Tullis, J. B., and C. T. Whittemore. 1980. Digestibility of fully hydrogenated tallow for growing pigs. Anim. Feed Sci. Tech, 5:87-91.

Vandermeers, A., M. C. Vandermeers-Piret, J. Rathe, J. Christophe. 1974. On human pancreatic triacylglycerol lipase: isolation and some properties. Biochim. Biophys. Acta. 370:257-268.

van Milgen, J., J. Noblet, and S. Dubois. 2001. Energetic efficiency of starch, protein, and lipid utilization in growing pigs. J. Nutr. 131:1309-1318.

Vaughan, M., J. E. Berger, and D. Steinberg. 1964. Hormone sensitive lipase and monoglyceride lipase activities in adipose tissue. J. Biol. Chem. 239:401-409.

Vernon, R. G. 1992. Effects of diet on lipolysis and its regulation. P. Nutr. Soc. 51:397-408.

Wang, A. M., M. V. Doyle, and D. F. Mark. 1989. Quantitation of mRNA by the polymerase chain reaction. Proc. Natl. Acad. Sci. U.S.A. 86:9717-21.

Wang, H., and R. H. Eckel. 2009. Lipoprotein lipase: from gene to obesity. Am. J. Physiol. Endocrinol. Metab. A review. 297:271-288.

Wang, L. F., M. L. Swift, and R. T. Zijlstra. 2012. Evaluation of energy digestibility of canola coproducts by in vitro analyses and characterization of fat digestion using spectroscopy. J. Anim. Sci. 90:251-253. doi:10.2527/jas.54000.

Wang, S. Y., W. Bottje, P. Maynard, J. Dibner, and W. Shermer. 1997. Effect of santoquin and oxidized fat on live and intestinal glutathione in broilers. Poult. Sci. 76:961-967.

Warnants, N., M. J. Van Oeckel, and C. V. Boucqué. 1999. Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues. J. Anim. Sci. 77:2478–2490. doi:10.2527/1999.7792478x

Watt, M. J., and A. J. Hoy. 2012. Lipid metabolism in skeletal muscle: generation of adaptive and maladaptive intracellular signals for cellular function. Am. J. Physiol. Endocrinol. Metab. 302:E1315-1328.

Westergaard, H., and J. M. Dietshcy. 1976. The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal muscle cell. J. Clin. Invest. 58:97-108.

Wiseman, J., D. J. A. Cole, and B. Hardy. 1990. The dietary energy values of soya-bean oil, tallow, and their blends for growing/finishing pigs. Anim. Prod. 50:513-518.

Wood, J. D., M. Enser, A. V. Fisher, G. R. Nute, P. R. Sheard, R. I. Richardson, S. I. Hughes, and F. M. Whittington. 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat. Sci. 78:343-58. doi:10.1016/j.meatsci.2007.07.019.

Wood, J. D., P. J. Buxton, F. M. Whittington, and M. Enser. 1986. The chemical composition of fat tissues in the pig: effects of castration and feeding treatment. Livest. Prod. Sci. 15:73–82.

Wood, J. D., C. D. Jones, J. A. Bayntun, and E. Dransfield. 1985. Backfat quality in boars and barrows at 90 kg live weight. Anim. Prod. 40:481–487.

Wu, F., L. J. Johnston, P. E. Urriola, and G. C. Shurson. 2016. Pork fat quality of pigs fed distillers dried grains with solubles (DDGS) with variable oil content and evaluation of iodine value prediction equations. J. Anim. Sci. 94:1041-1052. doi:10.2527/jas.2015-9593.

Young, S. G., and R. Zechner. 2013. Biochemistry and pathophysiology of intravascular and intracellular lipolysis. Genes. Dev. 27:459-484. doi:10.1101/gad.209296.112.

Zwicker, B. L., and L. B. Agellon. 2013. Transport and biological activities of bile acids. Int. J. Biochem. Cell. Biol. 45:1389-1398. doi:10.1016/j.biocel.2013.04.012.

Table 1.1. Identification of fatty acids found in this dissertation or commonly found in nature[1]

Common name	Number of carbons	Number of double bonds	Scientific name	n-	Common source
Formic	1	0	Methanoic acid	C1:0	insect stings
Acetic	2	0	Ethanoic acid	C2:0	vinegar
Propionic	3	0	Propanoic acid	C3:0	bacteria fermentation
Butyric	4	0	Butanoic acid	C4:0	butter fat
Valeric	5	0	Pentanoic acid	C5:0	flowering plants
Caproic	6	0	Hexanoic acid	C6:0	goat fat
Caprylic	8	0	Octanoic acid	C8:0	coconut oil
Capric	10	0	Decanoic acid	C10:0	coconut oil
Lauric	12	0	Dodecanoic acid	C12:0	coconut oil
Myristic	14	0	Tetradecanoic acid	C14:0	coconut oil
Myristoleic	14	1	9-Tetradecenoic acid	C14:1n-9	seed oil, ruminant animal fat
Pentadecanoic	15	0	Pentadecanoic acid	C15:0	butter fat
Palmitic	16	0	Hexadecanoic acid	C16:0	palm oil
Palmitoleic	16	1	9-Hexadecenoic acid	C16:1n-9	fish oil, animal fat
Hexadecadienoic	16	2	9,12-Hexadecenoic acid	C16:2n-4	fish oil
Margaric	17	0	Heptadecanoic acid	C17:0	butter fat
Margaroleic	17	1	9-Heptadecenoic acid	C17:1n-9	butter fat, fish oil
Stearic	18	0	Octadecanoic acid	C18:0	animal fat
Oleic	18	1	9-Octadecenoic acid	C18:1n-9	animal fat
Ricinoleic	18	1	12-Hydroxy-9-octadecenoic acid	C18:1n-9	castor oil
Vaccenic	18	1	11-Octadecenoic acid	C18:1n-7	butter fat
Linoleic	18	2	9,12-Octadecadienoic acid	C18:2n-6	cereal grain oil
α-Linolenic	18	3	9,12,15-Octadecatrienoic acid	C18:3n-3	flaxseed oil
γ-Linolenic	18	3	6,9.12-Octadecatrienoic acid	C18:3n-6	borage oil
Octadecatetraenoic	18	4	6,9,12,15-Octadecatetraenoic acid	C18:4n-3	fish oil
Nonadecenoic	19	1	10-Nonadecenoic acid	C19:1n-9	canola oil
Arachidic	20	0	Eicosanoic acid	C20:0	peanut oil
Gadoleic	20	1	9-Eicosanoic acid	C20:1n-11	fish oil
Eicosadienoic	20	2	11,14-Eicosadienoic acid	C20:2n-6	pork fat
homo-γ Linolenic	20	3	8,11,14-Eicosatrienoic acid	C20:3n-6	fish oil
Arachidonic (AA)	20	4	5,8,11,14-Eicosatetraenoic acid	C20:4n-6	liver fat
Eicosapentaenoic (EPA)	20	5	5,8,11,14,17-Eicosapentaenoic acid	C20:5n-3	fish oil
Behenic	22	0	Docosanoic acid	C22:0	rapeseed oil
Erucic	22	1	13-Docosenoic acid	C22:1n-9	rapeseed oil
Docosatrienoic	22	3	13,16,19-Docosatrienoic acid	C22:3n-3	fish oil
Docosatetraenoic	22	4	7,10,13,16-Docosatetraenoic acid	C22:4n-6	sphingolipids
Docosapentaenoic (DPA)	22	5	7,10,13,16,19- Docosapentaenoic acid	C22:5n-3	fish oil
Docosahexaenoic (DHA)	22	6	4,7,10,13,16,19-Docosahexaenoic acid	C22:6n-3	fish oil
Lignoceric	24	0	Tetracosanoic acid	C24:0	peanut oil
Nervonic	24	1	15-Tetracosenoic acid	C24:1n-9	sphingolipids

[1]Adapted from Scrimegour and Harwood (2007); Kerr et al. (2015).

Table 1.2. Lipases in the gastrointestinal tract of pig that facilitate in dietary fat digestion

Lipase	Location	Function/products of hydrolysis
Lingual	Mouth	hydrolysis of fatty acids from the sn[1]-3 position of triacylglycerides
Gastric	stomach	hydrolysis of short chain fatty acids from triacylglycerides
Colipase	duodenum to ileum	anchors pancreatic lipase to lipid droplets and shuttles hydrolyzed lipid products to micelles
Pancreatic	duodenum to ileum	hydrolysis of fatty acids from the sn-1 and sn-3 positions of triacylglycerides
Phospholipase A_2	duodenum to ileum	hydrolysis of fatty acids from the sn-2 position of phospholipids

[1]sn = stereochemcial number on the glycerol backbone.

Table 1.3. Characteristics of the major classes of lipoproteins[1]

Lipoprotein	Density (g/dL)	Diameter (nm)	Lipid, %		
			Triglyceride	Cholesterol	Phospholipid
Chylomicron	0.95	75 to 1200	80 to 95	2 to 7	3 to 9
Very low density lipoprotein	0.95 to 1.01	30 to 80	55 to 80	5 to 15	10 to 20
Intermediate density lipoprotein	1.01 to 1.02	25 to 35	25 to 35	20 to 40	15 to 25
Low density lipoprotein	1.02 to 1.06	18 to 25	5 to 15	40 to 50	20 to 25
High density lipoprotein	1.06 to 1.21	5 to 12	5 to 10	15 to 25	20 to 30

[1]Adapted from Jones and Rideout (2012) and Saunders and Ginsberg (1994).

Table 1.4. Estimations of endogenous losses of fat digestion (ELF) at the end of the ileum and over the entire intestinal tract

Source	BW (kg)	ELF at the ileum (g/kg of DMI[1])	ELF over the entire intestinal tract (g/kg of DMI)
Extracted fat			
Adams and Jensen, 1984	6	-	4.4
Jorgensen et al., 1993	75	4.74	4.41
Jorgensen and Fernandez, 2000	63	-	22.4
Kil et al., 2010	38	3.28	3.77
Kim et al., 2013	52	6.11	6.51
Intact fat			
Adams and Jensen, 1984	6	-	6.1
Adams and Jensen, 1985	10	-	8.7
Kil et al., 2010	38	7.27	12.08
Both extracted and intact fat			
Gutierrez et al., 2016	34	9.47	13.64

[1]DMI = Dry matter intake.

Table 1.5. Estimations of NE of dietary fat sources in the past 30 years

Source	BW (kg)	Dietary fat source	NE (Mcal/kg)
Cera et al., 1989	6 to 15	coconut oil	6.18[1]
Galloway and Ewan, 1989	6 to 10	Tallow	4.18
NRC, 1998	-	all sources	4.93 to 5.37
van Milgen et al., 2001	60	vegetable oil	7.02
Sauvant et al., 2004	-	all sources	7.12
Kil et al., 2011	22, 84[2]	soybean oil	4.68
Kil et al., 2011	22, 84[2]	choice white grease	5.90
NRC, 2012	-	all sources	6.18 to 7.55
Boyd et al., 2015	38 to 66	choice white grease	8.06
Boyd et al., 2015	79 to 107	choice white grease	8.50
Rosero et al., 2015	unknown[3]	animal-vegetable blend	7.17[1]
Rosero et al., 2015	unknown[3]	choice white grease	7.23[1]

[1]DE was converted to NE via NE = (DE × 98%) × 88% (van Milgen et al., 2001; NRC, 2012).
[2]Combined NE of the 2 reported BW.
[3]BW was not reported for the lactating sows.

Table 1.6. Digestibility experiments of dietary fat sources in pigs in the past 60 years

Source	BW (kg)	Dietary fat		Key finding(s)
		Source	Level, %	
Lloyd et al., 1957	NA[1]	short, medium, long[2]	20	ATTD[3] of short chain > medium chain > long chain; ATTD increased with age
Eusebio et al., 1965	NA	soybean oil, lard, tallow	2.5, 5, 10	ATTD increased with age; no difference among sources
Hamilton and McDonald, 1969	6, 18	coconut oil, rapeseed oil, lard, tallow	10	No differences among sources or BW; most fecal lipids are FFA[4] and saturated
Frobish et al., 1970	7, 11	butter, coconut oil, lard, vegetable oil, HF[5]	10	No differences among sources; ATTD increased with BW
Cera et al., 1988, 1989, 1990	6 to 16	corn oil, lard, tallow	8	ATTD of medium chain source > long chain unsaturated sources > long chain saturated sources
Jones et al., 1992	8	soybean oil, tallow, lard, coconut oil	10	ATTD of unsaturated fatty acids > saturated fatty acids
Jorgensen et al., 1993	75	soybean oil	0 to 3	Unsaturated fatty acids are hydrogenated by microbes in the large intestine
Jorgensen and Fernandez, 2000	60	blends of animal fat, soy oil, palm oil, and vegetable oil	5 to 30	Increased level of FFA decreased ATTD
Jorgensen et al., 2000	35	rapeseed oil, fish oil, coconut oil	15	No difference among sources or AID[6] and ATTD; ATTD of saturated fatty acid was lower than AID due to biohydrogenation
DeRouchey et al., 2004	13	choice white grease[7]	6	No difference among FFA level for ATTD
Averette Gatlin et al., 2005	62	soy oil and hydrogenated fat	5	ATTD of fully hydrogenated fat is near 0
Duran-Montge et al., 2007	45	tallow, sunflower oil, linseed oil	10	ATTD is ~6% greater than AID (more if saturated); AID increases with increased unsaturation and fatty acid chain length
Kil et al., 2010	38 to 97	corn oil	2, 4, 6	No difference between TID[8] and TTTD[9]
Kil et al., 2011	22, 84	choice white grease, soy oil	5, 10	No difference among sources
Adeola et al., 2013	11, 20	soybean oil, tallow	1, 3, 5	No difference among sources; ATTD increased as BW increased
Mendoza and van Heugten, 2014	9 to 21	blend[10]	6	ATTD decreased due to increased FFA level; ATTD decreased as the iodine value of the dietary fat increased
Rosero et al., 2015	NA	blend[10]	6	ATTD decreased due to increased FFA level, but negative impact was less as unsaturation of the dietary fat increased

[1]NA = not available.

[2]Short (included pens fed either butter or coconut oil), medium (included pens fed lard, tallow, linseed oil, or corn oil), long (included pens fed fish oil, rapeseed oil or erucic acid).

[3]ATTD = apparent total tract digestibility (%) of fat.

[4]FFA = free fatty acid (%).

[5]HF = hydrolyzed animal and vegetable fat; contained 40% FFA.

[6]AID = Apparent ileal digestibility (%) of fat.

[7]Source was artificially oxidized to create FFA and rancidity.

[8]TID = True ileal digestibility (%) of fat.

[9]TTTD = True total tract digestibility (%) of fat.

[10]Soybean oil, choice white grease, choice white acid grease, soybean-cottonseed acid-oil.

Table 1.7. Prediction equations for iodine value (IV) of carcass backfat, belly fat, jowl fat, and the average of the 3 fat depots[1]

Source	Equation (IV =)	R^2
Backfat		
Madsen et al., 1992	47.1 + 0.14 × IVP[2] intake/d	0.86
Boyd et al., 1997	52.4 + 0.315 × Diet IVP	-
Benz et al., 2011	51.946 + 0.2715 × Diet IVP	0.16
Benz et al., 2011	35.458 + 14.324 × Diet C18:2[3], %	0.73
Cromwell et al., 2011	64.5 + 0.432 × DDGS in diet, %	0.92
Estrada Restrepo, 2013	60.13 + 0.27 × Diet IVP	0.81
Estrada Restrepo, 2013	70.06 + 0.29 × DDGS[4] in diet, %	0.81
Kellner et al., 2014	55.06 + 0.256 × Diet IVP	0.93
Kellner et al., 2014	55.96 + 0.163 × C18:2 intake/d, g	0.90
Kellner et al., 2014	56.34 + 4.80 × Diet C18:2, %	0.92
Paulk et al., 2015	84.83 + (6.87 × I EFA) − (3.90 × F EFA) − (0.12 × I d) − (1.30 × F d) − (0.11 × I EFA × F d) + (0.048 × F EFA × I d) + (0.12 × F EFA × F d) − (0.0060 × F NE) + (0.0005 × F NE × F d) − (0.26 × BF)[5]	0.95
Belly fat		
Estrada Restrepo, 2013	58.32 + 0.25 × Diet IVP	0.81
Estrada Restrepo, 2013	67.35 + 0.26 × DDGS in diet, %	0.81
Kellner et al., 2014	55.39 + 0.236 × Diet IVP	0.93
Kellner et al., 2014	55.96 + 0.152 × 18:2 intake/d, g	0.93
Kellner et al., 2014	56.36 + 4.47 × Diet C18:2, %	0.95
Paulk et al., 2015	106.16 + (6.21 × I EFA) − (1.50 × F d) − (0.11 × I EFA × F d) − (0.012 × I NE) + (0.00069 × I NE × F d) − (0.18 × HCW) − (0.25 × BF)	0.94
Jowl fat		
Benz et al., 2011	56.479 + 0.247 × Diet IVP	0.32
Benz et al., 2011	47.469 + 10.111 × Diet C18:2, %	0.90
Estrada Restrepo, 2013	64.54 + 0.27 × Diet IVP	0.81
Estrada Restrepo, 2013	72.99 + 0.24 × DDGS in diet, %	0.81
Kellner et al., 2014	64.24 + 0.152 × Diet IVP	0.86
Kellner et al., 2014	64.28 + 0.102 × 18:2 intake/d, g	0.94
Kellner et al., 2014	64.60 + 2.99 × Diet C18:2, %	0.95
Paulk et al., 2015	85.50 + (1.08 × I EFA) + (0.87 × F EFA) − (0.014 × I d) − (0.050 × F d) + (0.038 × I EFA × I d) + (0.054 × F EFA × F d)- (0.0066 × I NE) + (0.071× I BW) − (2.19 × ADFI) − (0.29 × BF)	0.93
Average of 3 depots or unspecified		
Christensen, 1962 (IVP)	Diet IV × ether extract (%) × 0.10	-
Kellner et al., 2014	58.566 + 0.1393 × C18:2 intake/d, g	0.94
Kellner et al., 2014	58.102 + 0.2149 × Diet IVP	0.93

[1]Adapated from Wu et al. (2016).

[2]IVP = iodine value product (Christensen, 1962; Madsen et al., 1992).

[3]C18:2 = linoleic acid.

[4]DDGS = Distillers dried grains with solubles.

[5]I = initial diet, F = final diet, d = days of diet fed, EFA = essential fatty acids (C18:2 and linolenic acid; %), NE (kcal/kg), BW (kg), ADFI (kg), HCW (kg), and BF = backfat depth (mm).

Table 1.8. Dietary fat quality indices[1]

Item	Description
Acid value (AV)	Amount of KOH needed to neutralize organic acids (measurement of free fatty acids)
Butylated hydroanisole (BHA) or Butylated hydroxytolune (BHT)	Amount of BHA and BHT (antioxidants)
Capillary melting point	Melting point of fat/oil source
Color	Quantified relative to the Fat Analysis Committee (FAC) standard [1 (light) to 45 (dark)]
Ethoxyquin	Amount of ethoxyquin (antioxidant)
Fatty acid profile	Concentration of individual fatty acids
Free fatty acids (FFA)	Amount of fatty acids not bound to the glycerol backbone in a triglyceride
Insolubles (I)	Amount of sediment in a sample (fiber, hair, hide, bone, soil, etc...)
Iodine value (IV)	Measure of unsaturation (can also be calculated based upon fatty acid profile)
Metals	Amount of (Ca, Cu, Fe, Mg, Mn, Na, P, and Zn)
Moisture (M)	Amount of moisture in a sample
Mono, di, and/or triglycerides	Amount of lipid structure present
Nonelutable material (NEM)	Total amount of non-nutritional material (moisture, impurities, unsaponifiable material, glycerol, oxidized/polymerized fats)
Phospholipids (Lecithin)	Amount of phospholipids
Saponification value	An estimate of the average molecular weight of the constituent fatty acids in sample (milligrams of KOH required to saponify 1 gram of lipid)
Titer	The solidification point of fatty acids in lipids
Unsaponifiables (U)	Amount of material in the lipid (sterols, hydrocarbons, pigments, fatty alcohols, vitamins, etc…) that will saponify (form a soap) when mixed with caustic soda (NaOH or KOH)
Unsaturated:saturated ratio (U:S)	Ratio of unsaturated to saturated fatty acids (Calculated from a fatty acid profile)

[1]Adapated from Kerr et al. (2015).

Table 1.9. Dietary fat peroxidation indices[1]

Item	Description
2, 4-decadienal (DDE)	Measures the content of an aldehyde derived from the peroxidation of linoleic acid
4-hydroxynonenal (HNE)	Measures the content of an α, β-unsaturated lipophilic aldehyde formed from the peroxidation of polyunsaturated omega-6 fatty acids, such as linoleic or arachidonic acid
Active oxygen method stability (AOM)	A predictive method where purified air is bubbled through a lipid sample at 97.8°C, and the PV of the lipid is determined at regular intervals to determine the time required to reach a PV of 100 mEq/kg lipid (recorded as h), or the PV of the lipid is determined at a predetermined time endpoint, such as at 20 h (recorded as mEq/kg lipid)
Hexanal (HEX)	Quantifies major secondary lipid oxidation products produced from the termination phase during the oxidation of linoleic and other omega-6 fatty acids
Oil stability index (OSI)	A method where air passes through a lipid under a specific temperature, at which point volatile acids decomposed from lipid peroxidation are driven out by the air and subsequently dissolved in water thereby increasing its conductivity. The conductivity of the water is constantly measured, and the OSI value is defined as the hours required for the rate of conductivity to reach a predetermined level
p-Anisidine value (AnV)	Measures the amount of the high molecular weight saturated and unsaturated aldehydes
Peroxide value (PV)	Measures the content of lipid peroxides and hydroperoxides
Thiobarbituric acid reactive substance concentration (TBARS)	Measures the content of carbonyl-containing secondary lipid oxidation products formed from the decomposition of hydroperoxides. Developed to detect malondialdehyde, although other carbonyl compounds can also contribute to the TBARS values
Triacylglycerol dimers and polymers	Measures the polymeric compounds formed during the late phases of peroxidation; quantification of compounds based on molecular size using size exclusion chromatography or a relative value using viscosity

[1]Adapted from Kerr et al. (2015).

Figure 1.1 Current dogma of lipid digestion in the pig via pancreatic lipase, micelle formation, and passive absorption (Jones and Rideout, 2012)

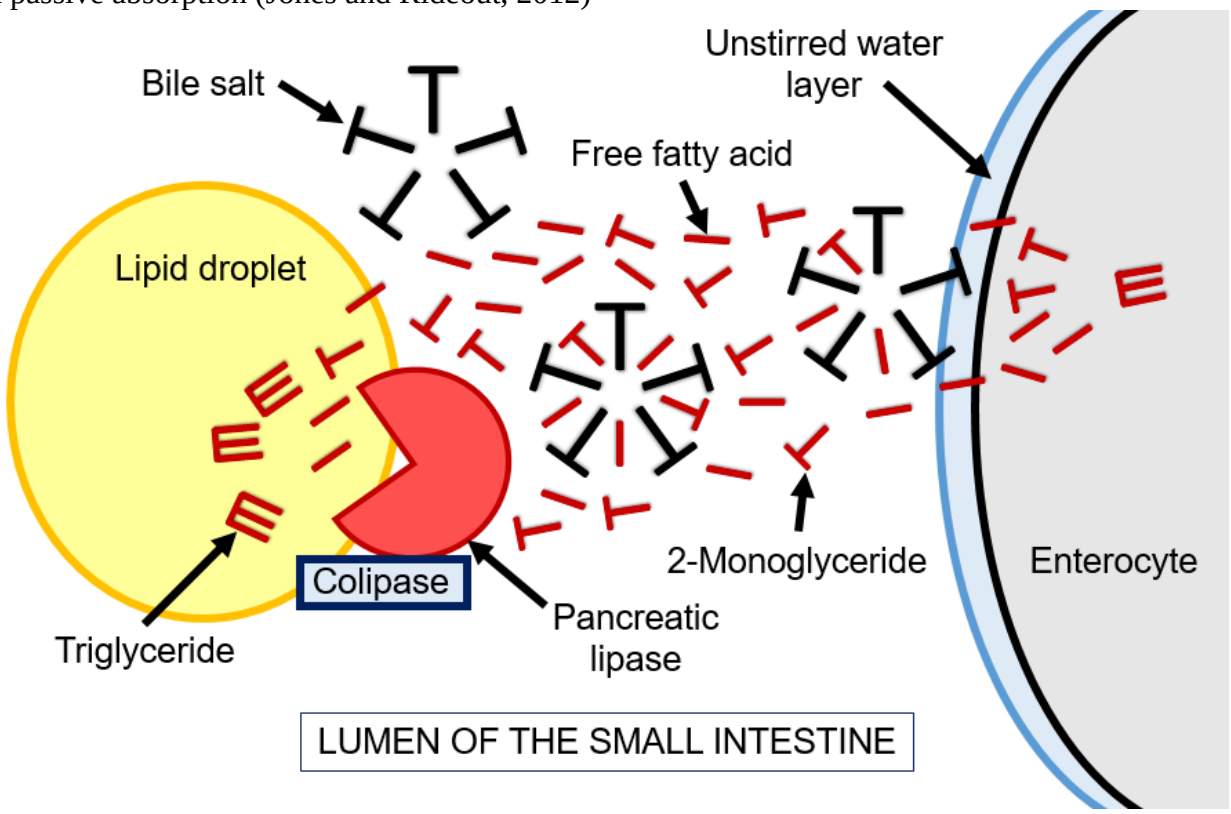

Figure 1.2. Simplified working theory of de novo lipogenesis in adipocytes of pigs

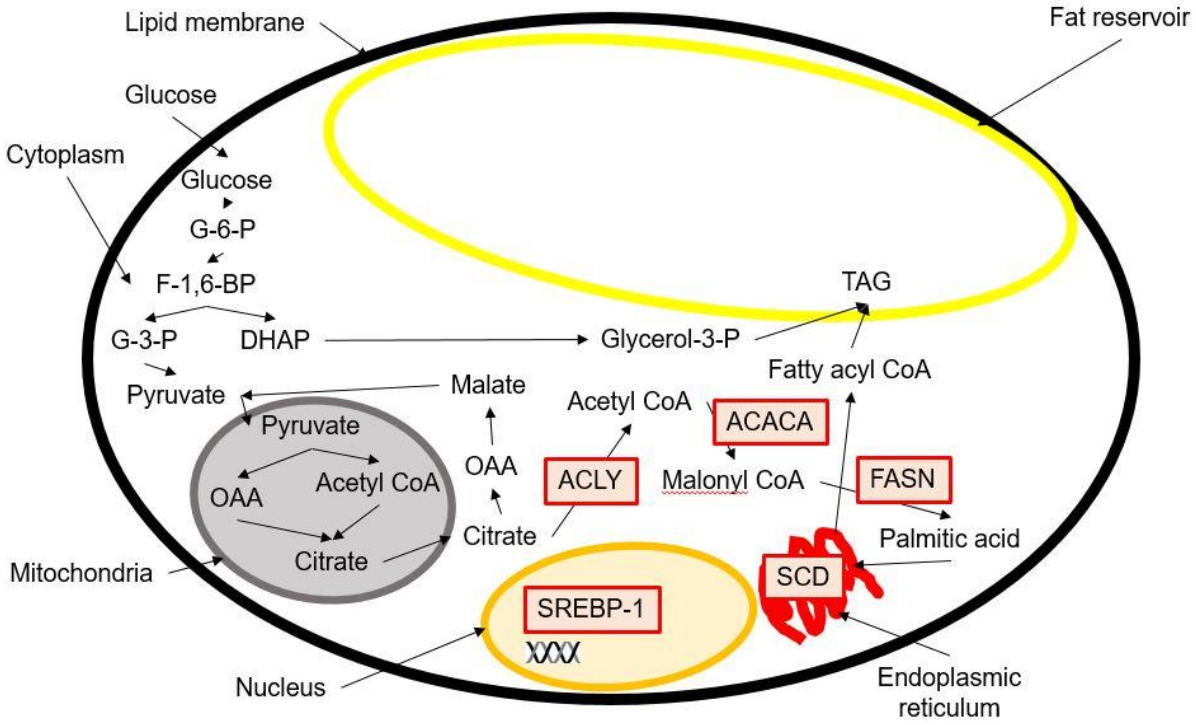

Figure 1.3. Simplified working theory of lipolysis in adipocytes tissue of pigs

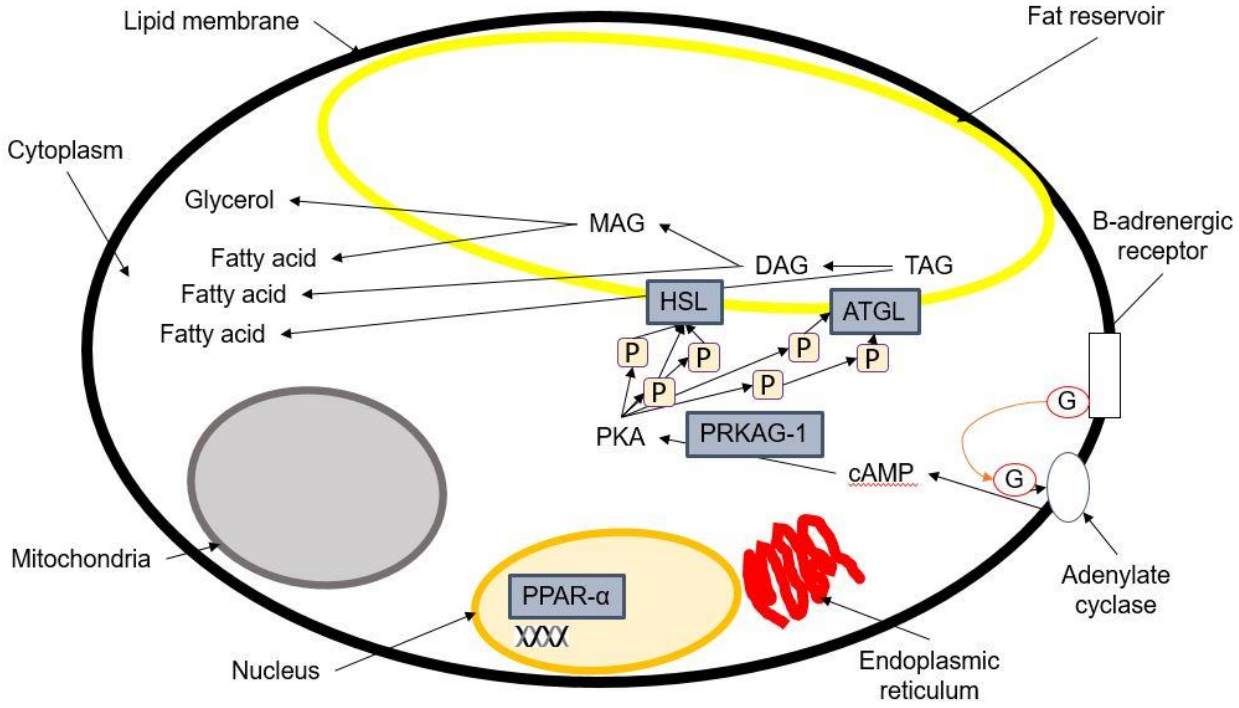

Figure 1.4. Example of growth assay to calibrate the NE value of dietary fat via a reference diet and a gain:feed ratio regression curve (Boyd et al., 2015)

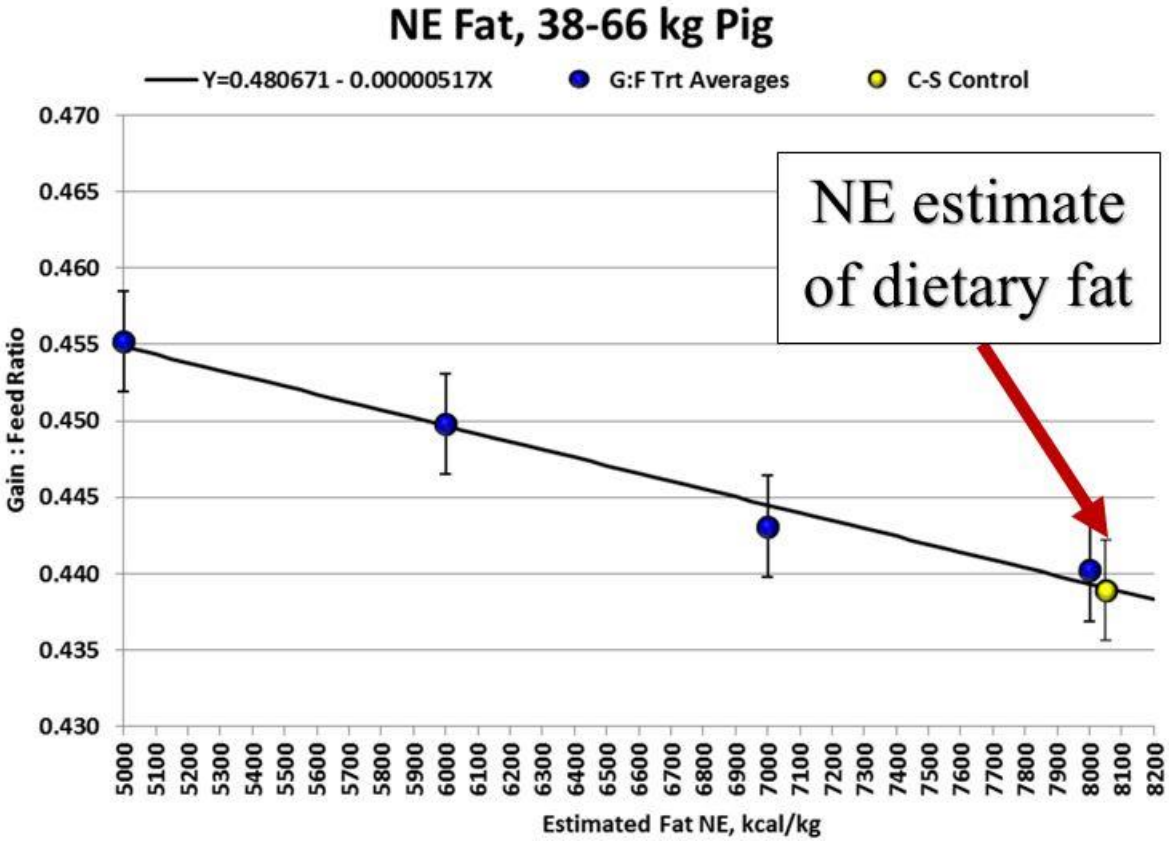

Figure 1.5. Simplified depiction of the lipid peroxidation process (Nawar, 1996)

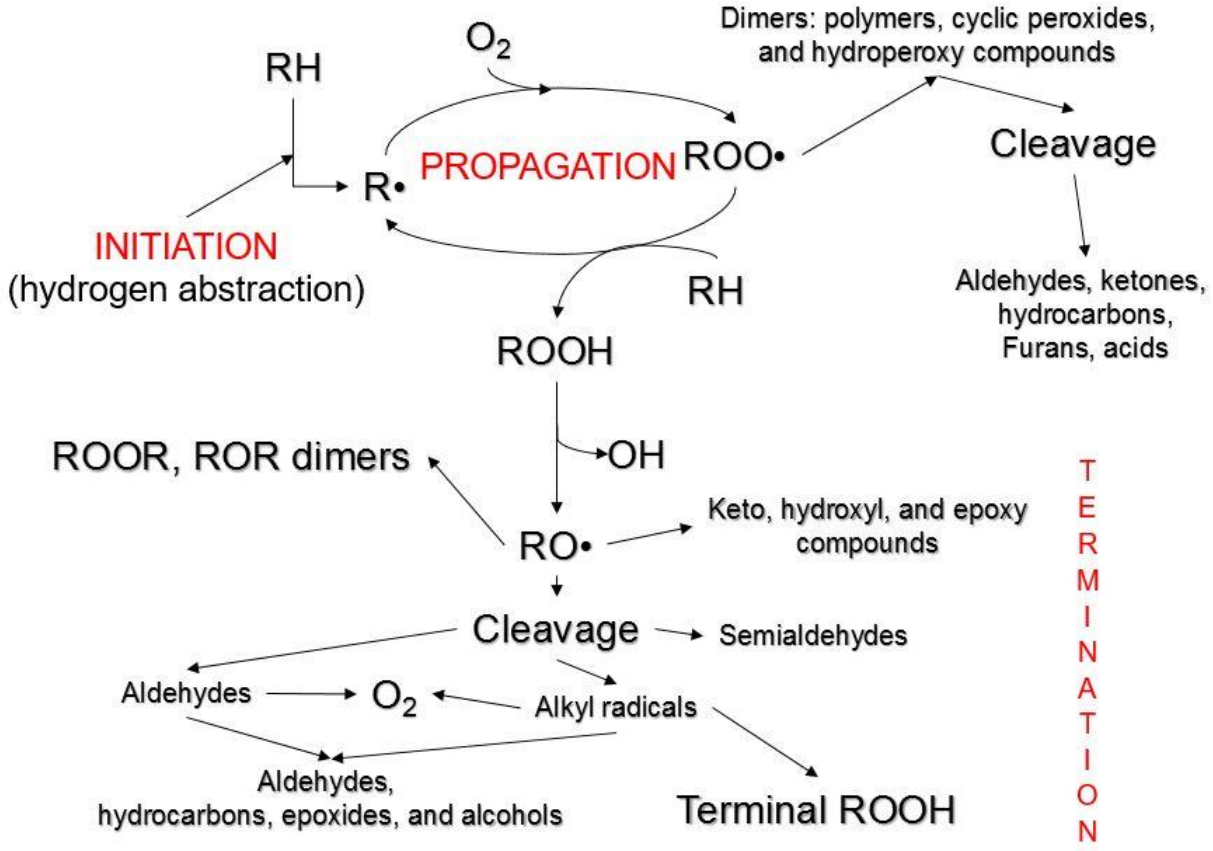

Figure 1.6. Range of dietary fat sources that can be included in swine diets, arranged by degree of unsaturation from top left (coconut oil [unsaturated to saturated fatty acid concentration ratio of 0.01]) to bottom right (canola oil [unsaturated to saturated fatty acid concentration ratio of 13.60])

CHAPTER II

PREDICTION OF PORCINE CARCASS IODINE VALUE BASED ON DIET COMPOSTION AND FATTY ACID INTAKE

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T. A. Kellner*, G. G. Gourley†, S. Wisdom‡, and J. F. Patience*

*Department of Animal Science, Iowa State University, Ames, 50011, USA; †Gourley Research Group, Webster City, IA, 50595, USA; and ‡Swine Graphics Enterprises, Webster City, IA, 50595, USA

Abstract

The pig industry utilizes a variety of fat sources (FS) and fat levels (FL) in diets to increase energy content. The objective was to investigate the impact of FS and FL on rate and efficiency of gain, apparent total tract digestion of dietary fat, pork fat composition, and test dietary predictors of carcass iodine value (IV). A total of 1,213 pigs (PIC 280 × PIC Camborough 42; PIC, Inc., Hendersonville, TN) with an initial BW of 32.0 ± 0.4 kg were allotted randomly to 1 of 6 dietary treatments on d 0. Treatments were arranged as a 2 × 3 factorial, with 2 FS: choice white grease [CWG (IV = 66.8)] or corn oil [COIL (IV = 123.2)] and 3 FL: 2, 4, or 6%. Ten pens of ~20 pigs each (0.70 m^2/pig) were randomly assigned to each of the 6 treatments. All pigs were on trial for 105 d. Pigs were harvested in 1 of 3 marketing pulls,

to achieve ideal market BW across differing rates of gain, at which time belly fat samples were collected [d 105 (457 pigs), d 117 (309 pigs), or d 134 (432 pigs)]. Diet and belly fat samples were analyzed for fatty acid profile. Daily rate of gain was not impacted by FS or FL ($P \leq$ 0.325). Increasing FL and dietary energy concentration increased G:F ($P < 0.001$). No difference was evident for G:F between FS ($P = 0.107$). Increasing FL of CWG resulted in greater daily intake of saturated fatty acids and monosaturated fatty acids than increasing FL of COIL ($P <$ 0.001). Increasing levels of COIL resulted in greater daily intake of polyunsaturated fatty acids than increasing levels of CWG ($P \leq 0.012$). Feeding CWG tended to result in great caloric efficiency adjusted for carcass yield than COIL ($P = 0.074$). The inclusion of COIL instead of CWG tended to increase true total tract digestion of acid hydrolyzed ether extract on d 39 ($P =$ 0.066), but not on d 104 ($P = 0.402$). Increasing COIL increased carcass IV at a greater magnitude than increasing CWG resulting in a FS × FL interaction on d 105, 117 and 134 ($P <$ 0.001). Dietary linoleic acid concentration and daily intake had a stronger linear relationship than IVP (iodine value product; $R^2 = 0.95$ vs. $R^2 = 0.94$ vs. $R^2 = 0.85$, respectively). In conclusion, limiting linoleic acid dietary concentration and intake is key to lowering carcass IV. To meet a carcass IV standard of 74 g/100 g, linoleic acid concentration had to be < 3.4% and intake had to be < 88 g/d. Dietary linoleic acid is a superior predictor of carcass IV compared to IVP, especially when high fat diets are used.

Introduction

The pig industry utilizes a variety of fat sources (**FS**) and fat levels (**FL**) in diets to increase energy content (Kerr et al., 2015). Dietary lipids provide linoleic (**C18:2**) and linolenic (**C18:3**) essential fatty acids to the pig (Cunnane, 1984; Palmquist, 2009). However, inclusion of

dietary fats and oils in swine diets is largely decided by economic factors (NRC, 2012). The primary economic factor driving usage is the cost/unit of energy provided (NRC, 2012).

A secondary economic factor driving usage of dietary lipids is the impact on carcass fat composition and quality (Semen et al., 2013). For 90 yr, it has been known that the nature of the lipid in the diet will be highly reflected in the composition of fat in the carcass (Ellis and Isbell, 1926). Therefore, it would seem highly probable that the lipid content of the diet could be used to predict the composition of carcass fat (Madsen et al., 1992; Boyd et al., 1997). Prediction of carcass iodine value (**IV**; a measurement of the degree of unsaturation of a lipid sample) was first attempted over 50 yr ago, by calculating the iodine value product (**IVP**) (Christensen, 1962; Madsen et al., 1992). Recently, IVP has been increasingly used to predict carcass IV (Benz et al., 2011, Wu et. al, 2016). Flaws of IVP have been identified (Kellner et al., 2014), and it has been suggested C18:2 concentration or intake (Benz et al., 2011; Kellner et al., 2014) or additional factors such as energy intake would be more accurate in predicting carcass IV (Paulk et al., 2015; Wu et al., 2016).

The objectives of this study were to investigate the impact of FS and FL on rate and efficiency of gain, apparent total tract digestion (**ATTD**) of dietary fat, and pork fat composition, to test dietary predictors of carcass IV. It was hypothesized that increasing FL would improve G:F, that FS would not impact G:F or digestion of dietary fat, and that dietary C18:2 concentration or intake would be a superior predictor of carcass IV than IVP.

Materials and methods

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research, and were approved by the Iowa State University Institutional Animal Care and Use Committee (number 10-14-7876-S).

Animals, Housing, and Experimental Design

A total of 1,213 pigs (PIC 280 × PIC Camborough 42; PIC, Inc., Hendersonville, TN) with an initial BW of 32.0 ± 0.4 kg were allotted randomly to 1 of 6 dietary treatments on d 0. Treatments were arranged as a 2 × 3 factorial, with 2 FS: choice white grease [**CWG** (IV = 66.8)] and corn oil [**COIL** (IV = 123.2)] and 3 inclusion levels of added dietary fat (2, 4, or 6%). Ten pens (5 pens of barrows and 5 pens of gilts) of ~20 pigs each (0.70 m^2/pig) were randomly assigned to 1 of the 6 treatments. Each of the 60 pens had slatted concrete floors, and were equipped with a stainless steel feeder and a trough drinker for ad libitum access to feed and water. These 60 pens were located in 1 of 2 identical rooms of 30 pens each. The trial was 134 d. Pigs were harvested in 1 of 3 marketing pulls on either d 105 (heaviest 457 pigs), d 117 (309 pigs), or d 134 (lightest 432 pigs) based on individual BW measured on d 105. Pigs harvested per pull was not equal across pens or treatment, but each pen had at least 2 pigs harvested per pull.

Diets and Feeding

All experimental diets (Table 2.1 – 2.4) were formulated to a constant ME and met or exceeded all nutrient requirements for pigs within this weight range (NRC, 2012). Diets contained 0.40% titanium dioxide in substitution of corn (from d 31 to 42 and d 95 to 105) to

allow determination of ATTD of acid hydrolyzed ether extract (**AEE**), DM, and GE. All diets were provided as a mash. Dietary fat sources (Table 2.5) were selected based on previous findings by Kellner et al. (2014) to provide a diverse range of resultant carcass IV to adequately compare predictors of carcass IV, while keeping in mind choices relevant to current production practices. Representative feed samples were collected at the time of mixing and biweekly from the feeder and stored at -20°C prior to analysis. Feed added to each pen was measured and distributed by an automated feed delivery system (FEEDPro, Feedlogic Corporation; Willmar, MN).

Sample Collection

Pigs were weighed individually on d 0 and 105. Pigs were weighed as a pen and feeders (feed depth) were measured on d 0, 21, 42, 63, 84, 105, 117, and 134 for determination of ADG and ADFI and calculation of G:F. Fecal grab samples were collected from a minimum of 3 pigs/pen on d 39 and 104, and immediately stored at -20°C for later analysis.

On d 105, 117, or 134 pigs (identified by a pen tattoo number) were harvested at a commercial packing plant (Tyson Foods Inc, Storm Lake, IA) where HCW was measured, and LM depth and 10th rib fat depth were estimated via ultrasound (Animal Ultrasound; Ithica, NY) on all carcasses before chilling. Samples of belly fat (subcutaneous, all layers, at the mid-line ranging from the 6th to 10th rib) from 2 carcasses per pen per marketing pull, for a total of 6 carcasses per pen and 60 carcasses per treatment were randomly collected, post deep chilling of carcasses, and stored at -20°C until analyzed.

Analytical Methods

Fatty acids were extracted from adipose tissue and feed samples by the 1-step direct transesterification procedure (Lepage and Roy, 1986). These samples were then assayed for total fatty acid content by gas chromatography (Model 3800 gas chromatograph, CP 8400 automatic injector, Varian Analytical Instruments, Walnut Creek, CA) using a 60 m × 0.25 mm column (Model DB-23, Agilent, Santa Clara, CA). Helium was utilized as a carrier gas at 0.5 mL/mm (1:50 split ratio). Oven temperature started at 50°C and increased to 235°C over a 26 min period. The injector and detector were maintained at 250°C. Identification of fatty acid peaks was performed by comparison with purified fatty acid samples from Sigma-Aldrich, Co. (St. Louis, MO).

Prior to analysis, fecal and feed samples were homogenized and then ground through a 1 mm screen in a Retsch grinder (model ZM1; Retsch Inc., Newtown, PA). Acid hydrolyzed ether extract (AEE; method 2003.06, AOAC International, 2007) was analyzed using a SoxCap SC 247 hydrolyzer and a Soxtec 255 semiautomatic extractor (FOSS North America, Eden Prairie, MN). Dry matter was determined according to a modified method (930.15, AOAC International, 2007) by drying samples in an oven at 105°C to a constant weight. Gross energy was determined using a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). Benzoic acid (6.318 Mcal/kg; Parr Instruments) was used as the standard for calibration (actual GE: 6.321 ± 0.005 Mcal/kg). Titanium dioxide was determined by spectrophotometer (synergy 4; BioTek, Winooski, VT) according to the method of Leone (1973). All chemical analyses were performed in duplicate and repeated when intra-duplicate CV was greater than 1%.

Calculations

Pig days were calculated as the number of pigs in each pen per day.

Iodine value of dietary fat samples, diet, and carcass fat samples was calculated from the fatty acid profile using the following equation: IV = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate percentage concentration (AOCS, 1998). The IVP of each diet was calculated as [IV of dietary lipids × ether extract (%)] × 0.10 (Christensen, 1962; Madsen et al., 1992). In addition, fatty acid intake (g/d) was calculated as ADFI (g/d) × dietary fatty acid (%) × dietary AEE (%; Kellner et al., 2014).

According to the equation of Oresanya et al. (2007), ATTD of AEE, DM, or GE were calculated as 100 – {100 × [concentration (g) of TiO_2 in diet × concentration of (g) of AEE, DM, or GE in feces]/[concentration (g) of TiO_2 in feces × concentration of AEE, DM, or GE in diet]}. True total tract digestibility (TTTD; %) of AEE was calculated via correcting ATTD of AEE for endogenous fat losses at 20 g of AEE/kg of DM intake.

Statistical Analysis

Analysis of the 6 treatments arranged as a 2 × 3 factorial, the main effects of FS (CWG vs. COIL) and FL (2 vs. 4 vs. 6%), and their interactions (**FS × FL**) was performed using PROC MIXED (SAS 9.4; SAS Inst. Inc., Cary, NC) with sex as a fixed effect and room as the random effect. Pen was the experimental unit. The comparison of final BW, carcass IV, and carcass measurements among marketing pulls (d 105 vs. 117 vs. 134) was modeled as an additional independent variable. For each variable, normal distribution of residuals was tested using PROC UNIVARIATE.

The comparison of the relationship between dietary fatty acid concentration or intake and fatty acid composition or IV of carcass belly fat was performed using PROC REG. Linear fit models were selected and reported based on having the best fit compared to quadratic and exponential fits. Multivariate models were tested via forward selection. Multivariate factors including ADG, ADFI, ME intake, days on feed, HCW, and backfat were selected and reported if they improved the relationship between C18:2 and carcass IV.

Non-detectable fatty acid values were treated in all statistical analyses as 0. All *P*-values less than 0.05 were considered significant and *P*-values between 0.05 and 0.10 were considered trends.

Results

Effect of Dietary Fat Source and Level, and Sex on Body Weight, Growth Performance, and Feed Efficiency

There were no interactions between FS and FL on ADG, ADFI, G:F, average market BW, and pig days per number of pigs sold ($P \geq 0.286$; Table 2.6). As expected, increasing FL increased G:F ($P < 0.001$) and decreased ADFI ($P = 0.028$), but did not impact ADG, average market BW, or pig days per number of pigs sold ($P \geq 0.417$). Dietary FS had no impact on ADG, ADFI, G:F, average market BW, and pig days per number of pigs sold ($P \leq 0.107$).

Barrows had increased ADFI and ADG, which resulted in less days on feed prior to market and an increase of average market BW than gilts ($P \geq 0.029$; Table 2.6). However, gilts had greater G:F ($P = 0.029$).

Effect of Dietary Fat Source and Level, and Sex on Fatty Acid and Energy Intake, and Caloric Efficiency

By design, increasing levels of CWG resulted in greater daily intake of the saturated fatty acids (**SFA**), namely palmitic (**C16:0**) and stearic (**C18:0**) and on the monounsaturated fatty acid (**MUFA**) oleic (**C18:1**) than of COIL, resulting in a FS × FL interaction ($P < 0.001$; Table 2.7). Also by design, increasing levels of COIL resulted in greater daily intake of the polyunsaturated fatty acids (**PUFA**) C18:2 and C18:3 than CWG, resulting in a FS × FL interaction ($P \leq 0.012$).

There were no interactions between FS and FL on energy intake or caloric efficiency ($P \geq 0.677$; Table 2.8). Feeding CWG at any level of inclusion tended to result in a greater caloric efficiency adjusted for carcass yield than COIL ($P = 0.074$), but not live BW caloric efficiency ($P = 0.109$) or ME intake ($P = 0.323$). Increasing FL had no impact on ME intake or caloric efficiency ($P \geq 0.350$).

In comparison to gilts, barrows had increased dietary fatty acid intake ($P < 0.001$; Table 2.7), and increased ME intake ($P < 0.001$; Table 2.8). Gilts were more efficient than barrows in converting dietary ME intake into BW and carcass weight gain ($P \leq 0.034$).

Effect of Dietary Fat Source and Level, and Sex on Digestibility of Dry Matter, Energy, and Lipids

A FS × FL interaction was evident on d 39 and 104 for ATTD of DM, GE, and AEE ($P \leq 0.013$; Table 2.9). Increasing COIL to 6% inclusion continued to increase ATTD of DM, GE, and AEE, while increasing CWG from 4% to 6% did not result in an increase. However, no interaction was evident between FS and FL for TTTD of AEE on d 39 and 104 ($P \geq 0.222$). The

inclusion of COIL instead of CWG tended to increase TTTD of AEE on d 39 ($P = 0.066$), but not on d 104 ($P = 0.402$).

Gilts had greater ATTD of DM ($P = 0.041$; Table 2.9), and tended to have greater ATTD of GE than barrows on d 39 ($P = 0.051$). On d 105, the sex effects on ATTD of DM and GE were not significant ($P \geq 0.292$). Sex did not impact ATTD or TTTD of AEE on d 39 or d 104 ($P \geq 0.484$).

Effect of Dietary Fat Source and Level, and Sex on Carcass Characteristics

There was no interaction between FS and FL on any carcass characteristic measured when pooled across all marketing pulls (d 105, 117, and 134; $P \geq 0.330$; Table 2.10). Inclusion of different FS had no impact on HCW ($P = 0.791$), yield ($P = 0.276$), backfat ($P = 0.180$), loin depth ($P = 0.826$), or percent lean ($P = 0.418$). Increasing the dietary FL tended to result in greater carcass yield ($P = 0.069$), but did not increase HCW ($P = 0.153$), backfat ($P = 0.287$), loin depth ($P = 0.670$), or percent lean ($P = 0.274$).

Gilts had decreased backfat, increased loin depth, and greater percent lean than barrows ($P \leq 0.002$; Table 2.10). Sex did not affect HCW or percent yield ($P \geq 0.190$).

Effect of Dietary Fat Source and Level on Fatty Acid Profile and Calculated Carcass Iodine Value

When all marketing pulls (d 105, 117, and 134) were pooled together (Table 2.11), it was evident that increasing dietary levels of COIL resulted in an linear increase while increasing dietary levels of CWG did not result in a linear increase for C18:2 and calculated IV, which resulted in a FS × FL interaction ($P < 0.001$). Similarly, there was a FS × FL interaction for

eicosadienoic acid (**C20:2**) concentration ($P = 0.046$) due to linear increase from increased COIL level and no such response in increased levels of CWG. Increased dietary levels of COIL decreased concentration of gadoleic (**C20:1**) and omega-3 to omega-6 fatty ratio while increased levels of CWG increased these resulting in a FS × FL interaction ($P < 0.001$). Increased levels of COIL resulted in a linear decrease response while CWG had no such linear decrease for concentrations of myristic acid (**C14:0**), C16:0, C18:0, and C18:1 resulting in a FS × FL interaction ($P \leq 0.039$). Increased dietary levels of COIL versus increased levels of CWG resulted in a greater dose response increase and a FS × FL interaction for MUFA palmitoleic acid (**C16:1**) and heptadecenoic acid (**C17:1**) ($P \leq 0.019$) and greater dose response decrease and a FS × FL interaction for PUFA C18:3 ($P < 0.005$). Inclusion of COIL rather than CWG increased concentrations of PUFA C18:2, C18:3, and C20:2 ($P \leq 0.005$), as well as increased calculated IV ($P < 0.001$) in belly fat samples pooled across all marketing pulls (d 105, 117, and 134). Reversely, inclusion of CWG rather than COIL tended to increased SFA lauric acid (**C12:0**) ($P = 0.078$) and tridecanoic acid (**C13:0**) concentrations ($P = 0.074$). Moreover, inclusion of CWG versus COIL increased the concentrations of SFA C14:0, C16:0, margaric acid (**C17:0**), and C18:0 ($P \leq 0.001$), MUFA C16:1, C17:1, C18:1, C20:1 ($P < 0.001$), and increased the omega-3 to omega-6 ratio ($P < 0.001$). Increasing dietary FL regardless of source increased PUFA concentrations of C18:2 and C18:3 ($P < 0.001$) and calculated IV ($P < 0.001$) in belly fat samples pooled across all marketing pulls (d 105, 117, and 134). Conversely, increased dietary FL decreased SFA concentrations of C10:0, C12:0, C16:0, C18:0, and arachidic acid (**C20:0**) ($P \leq 0.028$), MUFA concentrations of C16:1, C17:1, and C18:1 ($P \leq 0.007$), and the omega-3 to omega-6 fatty acid ratio ($P = 0.026$).

Effects of Sex on Fatty Acid Profile and Calculated Carcass Iodine Value

In carcass belly fat samples pooled across all marketing pulls (d 105, 117, and 134), barrows had increased concentrations of C16:0, C16:1, C17:1 and C20:1 ($P \leq 0.012$), and tended to have increased concentrations of C18:1 than gilts ($P = 0.099$; Table 2.11). Gilts had increased concentrations of C18:2 and C18:3 ($P < 0.001$). Carcass IV was greater in gilts than barrows ($P < 0.001$).

Differences in Carcass Characteristics and Carcass Iodine Value among Marketing Pulls

Pigs in latter marketing pulls (d 134 > d 117 > d 105) had an increase of average market BW ($P < 0.001$; Table 2.6). Pigs in latter marketing pulls had increased HCW, backfat, and loin depth, and decreased percent lean ($P < 0.001$; Table 2.10). There were no differences among marketing pulls (d 105 vs. 117 vs. 134) for carcass IV ($P = 0.899$; Table 2.11).

Comparison of Predictors of Carcass Iodine Value

Although, there was a linear relationship tendency for dietary C18:3 concentration to predict carcass IV ($P = 0.084$), out of all dietary fatty acids present, only dietary C18:2 concentration ($P < 0.001$) and intake ($P = 0.002$) had a significant linear relationship with carcass IV (Table 2.12). Dietary IVP also had a significant linear relationship with carcass IV ($P = 0.008$). However, both dietary C18:2 concentration and intake had a stronger linear relationship with carcass IV than dietary IVP ($R^2 = 0.95$ vs. $R^2 = 0.94$ vs. $R^2 = 0.85$). When adding additional variables to dietary C18:2 concentration with the linear relationship to carcass IV, only ME intake improved the precision of the relationship ($R^2 = 0.98$).

Discussion

The degree of unsaturation of pork fat is highly reflective of the degree of unsaturation of the fat source (Boyd et al., 1997; Apple et al., 2009). The biological reason for this is that the chemical structure of a dietary fatty acid is largely unaltered from consumption to deposition, a phenomenon which has been demonstrated in pigs for 100 yr (Ellis and Isbell, 1926; Allee et al., 1972). Due to this relationship, it is logical that the fat composition in the pig carcass can be predicted from the composition of the diet (Madsen et al., 1992; Boyd et al., 1997). The first attempt at such a prediction was reported over 50 yr ago resulted in the term IVP, a value that is based on an equation that includes both the IV concentration of the dietary fat and the level of fat in the diet times a constant of 0.10 (Christensen, 1962; Madsen et al., 1992).

Iodine value product is widely used in the pig industry as a tool to predict carcass IV. However, findings by Kellner et al. (2014) showed a flaw in the IVP equation that becomes particularly apparent when high fat diets are used. The weakness of the IVP equation arises from the fact that both diet IV and the inclusion level of dietary fat are weighted equally. Depending on which dietary fat source is employed and how saturated or unsaturated it is, a 2% dietary fat level increase may have little to no impact or a very large impact on carcass IV (Kellner et al., 2014). Results of the current experiment also exposes this weakness; for instance, the 6% CWG treatment with an IVP of 96.7, 97.0, 94.2, and 79.5 (phases 1 to 4, respectively) resulted in a carcass IV that was 4.9 g/100 g lower than the 2% COIL diet with an IVP of 92.2, 92.2, 91.1, and 71.7 (phases 1 to 4, respectively). Dietary C18:2 is not skewed by dietary fat level in its prediction of carcass IV, as it only factors in C18:2 and excludes other dietary fatty acids which do not have as strong a linear relationship with carcass IV; this is clearly shown by the data reported herein.

Another flaw of the IVP estimate is the inclusion of diet IV as a factor, because diet IV includes MUFA C16:1 and C18:1 (AOCS, 1998). These MUFAs can be sourced from the diet or be synthesized by the pig in the adipocyte via de novo lipogenesis (Kloareg et al., 2007). Consequently, the linear relationship between carcass IV and dietary C16:1 and C18:1 will not be as strong as dietary PUFAs such as C18:2 which can only be sourced from the diet. Therefore, diet IV and IVP has a weaker relationship with carcass IV than dietary C18:2.

It has been suggested that additional factors such as energy intake or carcass characteristics could increase the robustness of using dietary C18:2 as a predictor of carcass IV. In this particular data set, the dietary concentration of C18:2 could explain such a large portion of the variation in carcass IV that only the addition of ME intake to the prediction equation reduced the mean squared error and increased the R^2. Other factors like HCW, percent lean, backfat, caloric efficiency, and adding dietary C18:3 concentration did not have enough variation in this experiment to be significant predictors of carcass IV. Dietary C18:3 and other PUFAs that can only be sourced via the diet and not via de novo lipogenesis can and should be used as predictors of carcass IV in combination with C18:2, if the diet includes ingredients high in those fatty acids such as flaxseed and fish oil (NRC 2012; Paulk et al., 2015). A recent meta-analysis effort by Paulk et al. (2015) showed that including dietary C18:2, dietary C18:3, HCW, days on feed, backfat, and dietary energy concentration can be used to predict carcass IV, with dietary C18:2, dietary NE content, and carcass backfat thickness having the most impact on predicting carcass IV.

It is important to note that among the various attempts to predict carcass IV, the linear relationship between dietary fat composition and carcass fat composition is very strong and consistent (Wu et al., 2016). However, there is problem with such predictions. The IV of

carcasses from pigs grown in facilities in which pigs are housed individually, for example to measure daily feed intake or collect sequential blood samples (Kellner et al., 2014) will be lower than the carcass IV measured in pigs housed in a more commercial-like environment with larger pen groups and lower feed intake. The reduction of carcass IV in pigs that are individually housed, well managed, and of high health status is attributed to greater daily energy intakes that result in greater de novo lipogenesis rates than their counterparts in commercial production. In addition to the lower carcass IV, an increased slope and decreased y-intercept of the predictive regression equations of carcass IV have also been recorded (Kellner at el., 2014). This difference may create difficulty in evaluating different prediction equations in a single scenario or in applying prediction equations broadly. For example, to meet a carcass IV standard of 74 g/100 g (Semen et al., 2013), Kellner et al. (2014) found that maximum daily C18:2 intake needs to be less than 111 g/d, but the data reported herein indicate the maximum daily C18:2 intake needs to be less than 88 g/d, a 20% difference. Furthermore, it may be important to also note carcass fat sample location (Wiegand et al., 2011; Kellner et al., 2014), duration of feeding a dietary fats (Browne et al., 2013), the length of time of fat withdrawal from the diet prior to harvest (Xu et al., 2010, Asmus et al., 2014), dietary fat unsaturation load prior to switching or withdrawal of a fat source (Warnants et al., 1999; Kellner et al., 2015), diet form (Nemechek et al., 2013), and ractopamine inclusion (Weber et al., 2006; Apple et al., 2008). Clearly, these are all important factors that may influence results, but nonetheless, based on a number of publications, the use of C18:2 concentration appears to be superior to IVP (Benz et al., 2011; Kellner et al., 2014).

The decision to utilize dietary fat is primarily driven by the cost per unit of energy provided (NRC, 2012). A review by Patience (2012) concluded that while dietary energy

concentration is important, the best predictor of growth is daily energy intake. In this study, there were no differences in energy intake due to FS or FL, which is why neither impacted ADG. However, there was a trend for reduced ADFI in pigs fed higher energy (fat) diets, resulting in pigs consuming a similar quantity of energy per day (~8.74 Mcal ME). This combination of similar ADG and a tendency for altered ADFI due to pigs eating to constant energy value resulted in a G:F advantage with increasing dietary energy (fat) content.

Recent findings show that the response to daily energy intake is difficult to predict (Patience, 2012). Increasing dietary ME intake from 7.6 Mcal/d to 8.0 Mcal/d did not increase ADG or G:F in a study reported by Apple et al. (2009), while increasing ME intake from 8.0 Mcal/d to 8.38 Mcal/d did (Collins et al., 2009). Beaulieu et al. (2009) reported that an increase in ME intake from 8.68 Mcal/d to 9.10 Mcal/d resulted in improved G:F but not ADG. Moreover, diverse farm conditions create a wide range of daily energy intakes from: individually housed pigs (10.0 to 11.5 Mcal/d; Kellner et al., 2014) to group housed pigs in a commercial research farm as these data report (8.5 to 9.0 Mcal/d) to commercial production (6.5 to 8.0 Mcal/d; Patience, 2012).

Furthermore, there are a multitude of variables that impact feed efficiency (Patience et al., 2015), which causes the relationship between energy intake and G:F to be poorer than expected. Oresanya et al. (2008) reported an R^2 between NE intake and G:F of only 0.14; this occurred when the actual energy content of the diets was measured and not estimated, and daily feed intake, a large variable among group housed pigs, was recorded on an individual pig basis. Clearly more work is required to help understand the complex relationships between energy intake and rate and efficiency of gain, particularly at energy intake levels seen in commercial

production. This is especially true considering that energy is the most expensive component of the diet (Patience, 2012).

There were no differences between CWG and COIL for G:F, ME intake, or caloric efficiency on a live weight basis, suggesting that the energy available to the pig was similar between sources. However, there was a tendency for CWG to be more efficient in converting ME to carcass gain. A reasonable explanation for this is not immediately clear, but it could be the result of the numerical decrease in backfat thickness and the increase in percent lean among pigs fed CWG versus COIL. The energy cost of depositing protein is lower (10.0 kcal ME/g; Patience, 2012) than the energy cost of depositing lipid (11.7 kcal ME/g; van Milgen and Noblet, 2003; Barea et al., 2010; Patience, 2012). Thus, the leaner carcass will be more efficient metabolically than a fatter carcass. Furthermore, an unsaturated dietary fat source, versus a saturated dietary fat source, has been shown to increase mRNA abundance of fatty acid synthase, the rate limiting step of de novo lipogenesis (Duran-Montge et al., 2009). Based on this evidence, it would make sense that COIL fed pigs would be fatter and less efficient users of calories than their CWG-fed contemporaries.

Dietary lipids are highly digestible constituents of the diet (Cera et al., 1988; Jorgensen et al., 1996). In general, the ATTD of AEE of saturated dietary fat sources are less digested than unsaturated sources (Wiseman et al., 1990; Jorgensen et al, 2000) as evident on d 39, but the ATTD of AEE response to degree of saturation is not always consistent (Jorgensen and Fernandez, 2000; Kerr et al., 2009) as evident on d 104. Recently, investigators have brought into question the “true” digestion of dietary fats and oils (NRC, 2012). The true digestibility of dietary fat accounts for endogenous losses: lipids that are present in the feces from such sources as sloughed intestinal cells, secretions of bile salts, and microbial lipid mass. Current estimates

range from as low as 4 g of endogenous fat losses/kg of DM intake to 30 g of endogenous fat losses/kg of DM intake (Kil et al., 2010). Data generated recently by Acosta et al. (2015) range from 18 g endogenous fat losses/kg of DM intake to 22 g endogenous fat losses/kg of DM intake, and Gutierrez et al. (2015) reported 14 g of endogenous fat losses/kg of DM intake. By correcting ATTD of AEE for endogenous losses, these data showed that the differences in TTTD between FS and among FL are minimal or at the very least not to the degree of magnitude that ATTD of AEE presents. Using this approach, Kil et al. (2010) and Acosta et al. (2015) reported that the TTTD of endogenous fat present in ingredients, and added fat from pure fat sources is similar.

In conclusion, ADG and G:F were similar between the two fat sources, but CWG tended to be slightly more efficient in producing carcass gain than COIL. Increasing FL 1% and dietary energy concentration 0.09 Mcal/kg increased G:F by 0.007, but did not improve ADG or caloric efficiency. Limiting C18:2 dietary concentration or intake is key to lowering carcass IV. Under these experimental conditions, to meet a carcass IV standard of 74 g/100 g (Semen et al., 2013) the minimum dietary concentration of C18:2 had to be less than 3.4% and daily 18:2 intake had to be less than 88 g/d. Furthermore, dietary C18:2 is a superior predictor of carcass IV compared to IVP, especially when high fat diets are used.

Literature cited

Acosta, J. A., R. D. Boyd, and J. F. Patience. 2015. Endogenous and exogenous fat digestion in growing pigs. J. Anim. Sci. 93(Suppl. 2):75.

Allee, G. L., D. R. Romsos, G. A. Leveille, and D. H. Baker. 1972. Lipogenesis and enzymatic activity in pig adipose tissue as influenced by source of dietary fat. J. Anim. Sci. 35:41–47. doi:10.2527/jas1972.35141x.

American Oil Chemists' Society (AOCS). 1998. Recommended practice Cd 1c-85. In: Official methods and recommended practices of the AOCS. 5th ed. AOCS, Campaign, IL. p. 1.

Apple, J. K., C. V. Maxwell, D. L. Galloway, S. Hutchison, and C. R. Hamilton. 2009. Interactive effects of dietary fat source and slaughter weight in growing-finishing swine: II. Fatty acid composition of subcutaneous fat. J. Anim. Sci. 87:1423–1440. doi:10.2527/jas.2008-1454.

Apple, J. K., C. V. Maxwell, B. R. Kutz, L. K. Rakes, J. T. Sawyer, Z. B. Johnson, T. A. Armstrong, S. N. Carr, and P. D. Matzat. 2008. Interactive effect of ractopamine and dietary fat source on pork quality characteristics of fresh pork chops during simulated retail display. J. Anim. Sci. 86:2711–2722. doi:10.2527/jas.2007-0327.

Asmus, M. D., M. A. Tavarez, M. D. Tokach, S. S. Dritz, A. L. Schroeder, J. L. Nelssen, R. D. Goodband, and J. M. Derouchey. 2014. The effects of immunological castration and corn dried distillers grains with solubles withdrawal on growth performance, carcass characteristics, fatty acid analysis, and iodine value of pork fat depots. J. Anim. Sci. 92:2116–2132. doi:10.2527/jas.2013-6910.

Barea, R., S. Dubios, H. Gilbert, P. Sellier, J. van Milgen, and J. Noblet. 2010. Energy utilization in pigs selected for high and low residual feed intake. J. Anim. Sci. 88:2062–2072. doi:10.2527/jas.2009-2395.

Beaulieu, A. D., N. H. Willams, and J. F. Patience. 2009. Response to dietary digestible energy concentration in growing pigs fed cereal grain-based diets. J. Anim. Sci. 87:965–976. doi:10.2527/jas.2007-0834.

Benz, J. M., M. D.Tokach, S. S. Dritz, J. L. Nelssen, J. M. DeRouchey, R. C. Sulabo, and R. D. Goodband. 2011. Effects of dietary iodine value product on growth performance and carcass fat quality of finishing pigs. J. Anim. Sci. 89:1419–1428. doi:10.2527/jas.2010-3126.

Boyd, R. D., M. E. Johnston, K. Scheller, A. A. Sosnicki, and E. R. Wilson. 1997. Relationship between dietary fatty acid profile and body fat composition in growing pigs. PIC Technical Memo 153. PIC, Franklin, KY.

Browne, N. A., J. K. Apple, B. E. Bass, C. V. Maxwell, J. W. S. Yancey, T. M. Johnson, and D. L. Galloway. 2013. Alternating dietary fat sources for growing-finishing pigs fed dried distillers grains with solubles: I. Growth performance, pork carcass characteristics, and fatty acid composition of subcutaneous fat depots. J. Anim. Sci. 91:1493–1508. doi:10.2527/jas.2012-5528.

Cera, K. R., D. C. Mahan, and G. A. Reinhart. 1988. Weekly digestibilities of diets supplemented with corn oil, lard, or tallow by weanling swine. J. Anim. Sci. 66:1430–1437. doi:10.2527/jas1988.6661430x.

Christensen, K. D. 1962. Foderfedtets indflydelse pa smorrets og flaeskets kvalitet. (In Danish.) Thesis, Kgl. Vet.- og Landbohojsk., Copenhagen, Denmark.

Collins, C. J., A. C. Philpotts, and D. J. Henman. 2009. Improving growth performance of finisher pigs with high fat diets. Anim. Prod. Sci. 49:262–267. doi:10.1071/EA08243.

Cunnane, S. C. 1984. Essential fatty acid/mineral interactions with reference to the pig. In: J. Wiseman, editor, Fats in animal nutrition. Butterworths, London, UK. p. 167–183. doi:10.1016/B978-0-408-10864-5.50014-0.

Duran-Montgé, P., P. K. Theil, C. Lauridsen, and E. Esteve-Garcia. 2009. Dietary fat source affects metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. Animal 3:535–542. doi:10.1017/S1751731108003686.

Ellis, N. R., and H. S. Isbell. 1926. Soft pork studies. II. The influence of the character of the ration upon the composition of the body fat of hogs. J. Biol. Chem. 59:219–248.

Gutierrez, N. A., N. V. L. Serao, and J. F. Patience. 2015. Effects of reduced oil distillers dried grains with solubles and soybean oil on dietary lysine, fat, and fiber digestibility in corn based diets fed to growing pigs. J. Anim. Sci. 93(Suppl. 2):45.

Horwitz, W., and G. W. Latimer. 2007. Official methods of analysis. 18th rev. ed. AOAC Int., Gaithersburg, MD. Jorgensen, H., and J. A. Fernandez. 2000. Chemical composition and energy value of different fat sources for growing pigs. Acta Agric. Scand., Sect. A 50:129–136.

Jorgensen, H., V. M. Gabert, M. S. Hedemann, and S. K.Jensen. 2000. Digestion of fat does not differ in growing pigs fed diets containing fish oil, rapeseed oil or coconut oil. J. Nutr. 130:852–857.

Jorgensen, H., S. K.Jensen, and B. O. Eggum. 1996. The influence of rapeseed oil on digestibility, energy metabolism, and tissue fatty acid composition in pigs. Acta Agric. Scand., Sect. A 46:65–75.

Kellner, T. A., K. J. Prusa, and J. F. Patience. 2014. Impact of dietary fat source and concentration and daily fatty acid intake on the composition of carcass fat and iodine value sampled in three regions of the pork carcass. J. Anim. Sci. 92:5485–5495. doi:10.2527/jas.2014-7567.

Kellner, T. A., K. J. Prusa, and J. F. Patience. 2015. The impact of dietary fat withdrawal on carcass iodine value, belly characteristics, and changes in body fat over time. J. Anim. Sci. 93:247– 257. doi:10.2527/jas.2014-7650.

Kerr, B. J., T. A. Kellner, and G. C. Shurson. 2015. Characteristics of lipids and their feeding value in swine diets. J. Anim. Sci. Biotechnol. 6:30. doi:10.1186/s40104-015-0028-x.

Kerr, B. J., T. E. Weber, W. A. Dozier III, and M. T. Kidd. 2009. Digestible and metabolizable energy content of crude glycerin originating from different sources in nursery pigs. J. Anim. Sci. 87:4042–4049. doi:10.2527/jas.2008-1676.

Kil, D. Y., T. E. Sauber, D. B. Jones, and H. H. Stein. 2010. Effect of the form of dietary fat and the concentration of dietary neutral detergent fiber on ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. J. Anim. Sci. 88:2959–2967. doi:10.2527/jas.2009-2216.

Kloareg, M., J. Noblet, and J. van Milgen. 2007. Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. Br. J. Nutr. 97:35–44. doi:10.1017/S0007114507205793.

Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. J. Assoc. Off. Anal. Chem. 56:535–537.

Lepage, G., and C. Roy. 1986. Direct transesterification of all classes of lipids in a one-step reaction. J. Lipid Res. 27:114–120.

Madsen, A., K. Jacobsen, and H. P. Mortensen. 1992. Influence of dietary fat on carcass fat quality in pigs. A review. Acta Agric. Scand., Sect. A 42:220–225.

Nemechek, J., M. Tokach, J. DeRouchey, S. Dritz, R. Goodband, and J. Nelssen. 2013. Effects of diet form and fiber level before marketing on growth performance, carcass yield, and iodine value of finishing pigs. J. Anim. Sci. 91(Suppl. 2):74. (Abstr.)

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Oresanya, T. F., A. D. Beaulieu, E. Beltranena, and J. F. Patience. 2007. The effect of dietary energy concentration and total lysine/ digestible energy ratio on the growth performance of weaned pigs. Can. J. Anim. Sci. 87:45–55. doi:10.4141/A05-064.

Oresanya, T. F., A. D.Beaulieu, and J. F.Patience. 2008. Investigations of energy metabolism in weanling barrows: The interaction of dietary energy concentration and daily feed (energy) intake. J. Anim. Sci. 86:348–363. doi:10.2527/jas.2007-0009.

Palmquist, D. L. 2009. Omega-3 fatty acids in metabolism, health and nutrition and for modified animal product foods. Prof. Anim. Sci. 25:207–249. doi:10.15232/S1080-7446(15)30713-0.

Patience, J. F. 2012. The influence of dietary energy on feed efficiency in grow-finish swine. In: J. F. Patience, editor, Feed efficiency in swine. Wageningen Academic Publishers, Wageningen, the Netherlands. p. 101–129. doi:10.3920/978-90-8686-756-1_5.

Patience, J. F., M. C. Rossoni-Serao, and N. A. Gutierrez. 2015. A review of feed efficiency in swine: Biology and application. J. Anim. Sci. Biotechnol. 6:33. doi:10.1186/s40104-015-0031-2.

Paulk, C. B., J. R. Bergstrom, M. D. Tokach, S. S. Dritz, D. D. Burnett, E. W. Stephenson, M. A. Vaughn, J. M. DeRouchey, R. D. Goodband, J. L. Nelssen, and J. M. Gonzalez. 2015. Equations generated to predict iodine value of pork carcass back, belly, and jowl fat. J. Anim. Sci. 93:1666–1678. doi:10.2527/jas.2014-8400.

Semen, D. L., W. N. Barron, and M. Matzinger. 2013. Evaluating the ability to measure pork fat quality for the production of commercial bacon. Meat Sci. 94:252–256. doi:10.1016/j.meatsci.2013.01.009.

van Milgen, J., and J. Noblet. 2003. Partitioning of energy intake to heat, protein and fat in growing pigs. J. Anim. Sci. 81(Suppl. 2):86–93.

Warnants, N., M. J. Van Oeckel, and C. V. Boucqué. 1999. Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues. J. Anim. Sci. 77:2478–2490.

Weber, T. E., B. T. Richert, M. A. Belury, Y. Gu, K. Enright, and A. P. Schinckel. 2006. Evaluation of the effects of dietary fat, conjugated linoleic acid, and ractopamine on growth performance, pork quality, and fatty acid profiles in genetically lean gilts. J. Anim. Sci. 84:720–732.

Wiegand, B. R., R. B. Hinson, M. J. Ritter, S. N. Carr, and G. L.Allee. 2011. Fatty acid profiles and iodine value correlations between 4 carcass fat depots from pigs fed varied combinations of ractopamine and energy. J. Anim. Sci. 89:3580–3586. doi:10.2527/jas.2010-3303.

Wiseman, J., D. J. A. Cole, and B. Hardy. 1990. The dietary energy values of soy-bean oil, tallow, and their blends for growing/finishing pigs. Anim. Prod. 50:513–518. doi:10.1017/S0003356100005006.

Wu, F., L. J.Johnston, P. E. Urriola, and G. C. Shurson. 2016. Pork fat quality of pigs fed distillers dried grains with solubles (DDGS) with variable oil content and evaluation of iodine value prediction equations. J. Anim. Sci. 94:1041–1052. doi:10.2527/jas.2015-9593.

Xu, G., S. K. Baidoo, L. J. Johnston, D. Bibus, J. E. Cannon, and G. C. Shurson. 2010. Effects of feeding diets containing increasing content of corn distillers dried grains with solubles to grower-finisher pigs on growth performance, carcass composition, and pork fat quality. J. Anim. Sci. 88:1394–1410. doi:10.2527/jas.2008-1404.

Table 2.1. Ingredient and nutrient composition (as-fed basis) of the experimental diets, d 0 to 21

	Corn oil			Choice white grease		
Item	2%	4%	6%	2%	4%	6%
Ingredient, %						
Corn	48.61	45.60	42.24	48.61	45.60	42.24
Dried distillers grains with solubles	30.00	30.00	30.00	30.00	30.00	30.00
Soybean meal (46.5% CP)	17.16	18.17	19.55	17.16	18.17	19.55
Corn oil	2.00	4.00	6.00	-	-	-
Choice white grease	-	-	-	2.00	4.00	6.00
Monocalcium phosphate	1.28	1.27	1.25	1.28	1.27	1.25
Lysine sulphate (54.6%)	0.40	0.41	0.40	0.40	0.41	0.40
Salt	0.36	0.37	0.37	0.36	0.37	0.37
Vitamin and trace mineral premix[1]	0.15	0.15	0.15	0.15	0.15	0.15
Copper chloride	0.02	0.02	0.02	0.02	0.02	0.02
Optiphos 1000[2]	0.004	0.004	0.004	0.004	0.004	0.004
Analyzed composition						
DM, %	89.2	89.5	89.6	89.1	89.4	89.7
GE, Mcal/kg	4.23	4.32	4.41	4.21	4.30	4.43
Acid hydrolyzed ether extract, %	7.67	9.00	10.22	7.02	9.05	10.55
Diet IV[3], g/100g	120.2	120.4	118.4	103.5	94.8	91.9
IVP[4]	92.2	108.4	121.0	72.7	85.8	96.7
Fatty acid[5], %						
C16:0	16.0	17.0	17.1	18.9	23.2	21.2
C18:0	2.7	2.5	2.9	6.4	8.3	9.1
C18:1	25.5	24.8	25.8	28.4	30.3	32.5
C18:2	53.7	53.5	52.3	43.8	36.4	33.5
C18:3	1.7	1.7	1.7	1.5	0.9	1.2
Other	0.4	0.5	0.4	1.0	1.0	2.6
Omega-3 to omega-6 ratio	0.032	0.032	0.033	0.034	0.025	0.036
Calculated composition						
ME, Mcal/kg	3.36	3.45	3.54	3.36	3.45	3.54

[1]Provides 2,937 IU vitamin A, 734 IU vitamin D, 14 IU vitamin E, 1.5 mg menadione, 14.7 mg vitamin B_{12}, 2.2 mg riboflavin, 18.4 mg niacin, 11.0 mg pantothenic acid, 257.2 mg Co (cobalt carbonate), 6.6 g Cu (copper sulfate), 147.0 mg I (calcium iodine), 73.4 g iron Fe (iron sulfate), 20.3 g Mn (manganese sulfate), 0.198 mg Se (sodium selenite), 73.4 g Zn (zinc sulfate) per kilogram of diet.

[2]Huvepharma, Inc., Peachtree City, GA.

[3]Iodine value calculated from fatty acid composition: (IV) = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate percentage concentration (AOCS, 1998).

[4]Iodine value product (IVP) = [IV of dietary lipids × ether extract (%)] × 0.10 (Christensen, 1962; Madsen et al., 1992).

[5]Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3).

Table 2.2. Ingredient and nutrient composition (as-fed basis) of the experimental diets, d 21 to 42

	Corn oil			Choice white grease		
Item	2%	4%	6%	2%	4%	6%
Ingredient, %						
Corn[1]	53.46	50.63	47.80	53.46	50.63	47.80
Dried distillers grains with solubles	30.00	30.00	30.00	30.00	30.00	30.00
Soybean meal (46.5% CP)	12.38	13.22	14.05	12.38	13.22	14.05
Corn oil	2.00	4.00	6.00	-	-	-
Choice white grease	-	-	-	2.00	4.00	6.00
Monocalcium phosphate	1.27	1.26	1.25	1.27	1.26	1.25
Lysine sulphate (54.6%)	0.35	0.36	0.36	0.35	0.36	0.36
Salt	0.36	0.37	0.37	0.36	0.37	0.37
Vitamin and trace mineral premix[2]	0.15	0.15	0.15	0.15	0.15	0.15
Copper chloride	0.02	0.02	0.02	0.02	0.02	0.02
Analyzed composition						
DM, %	89.2	89.3	89.5	89.1	89.3	89.7
GE, Mcal/kg	4.19	4.26	4.33	4.14	4.27	4.36
Acid hydrolyzed ether extract, %	7.67	8.97	9.99	7.01	9.01	10.54
Diet IV[3], g/100g	120.2	120.4	118.3	103.5	94.7	91.9
IVP[4]	92.2	108.4	120.9	72.7	85.7	97.0
Fatty acid[5], %						
C16:0	15.9	16.1	16.3	20.5	21.6	20.5
C18:0	2.7	2.5	2.9	6.2	7.8	9.0
C18:1	25.3	25.1	26.0	28.5	31.7	32.6
C18:2	54.2	54.3	52.7	42.8	37.1	33.9
C18:3	1.7	1.7	1.7	1.5	1.0	1.2
Other, %	0.2	0.4	0.4	0.8	0.8	2.6
Omega-3 to omega-6 ratio	0.031	0.031	0.032	0.035	0.027	0.035
Calculated composition						
ME, Mcal/kg	3.36	3.45	3.54	3.36	3.45	3.54

[1]Titanium dioxide was included at 0.40% as an indigestible marker in substitution of corn from d 31 to 42.

[2]Provides 2,937 IU vitamin A, 734 IU vitamin D, 14 IU vitamin E, 1.5 mg menadione, 14.7 mg vitamin B_{12}, 2.2 mg riboflavin, 18.4 mg niacin, 11.0 mg pantothenic acid, 257.2 mg Co (cobalt carbonate), 6.6 g Cu (copper sulfate), 147.0 mg I (calcium iodine), 73.4 g iron Fe (iron sulfate), 20.3 g Mn (manganese sulfate), 0.198 mg Se (sodium selenite), 73.4 g Zn (zinc sulfate) per kilogram of diet.

[3]Iodine value calculated from fatty acid composition: (IV) = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate percentage concentration (AOCS, 1998).

[4]Iodine value product (IVP) = [IV of dietary lipids × ether extract (%)] × 0.10 (Christensen, 1962; Madsen et al., 1992).

[5]Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3).

Table 2.3. Ingredient and nutrient composition (as-fed basis) of the experimental diets, d 42 to 63

	Corn oil			Choice white grease		
Item	2%	4%	6%	2%	4%	6%
Ingredient, %						
Corn	56.72	53.99	51.26	56.72	53.99	51.26
Dried distillers grains with solubles	30.00	30.00	30.00	30.00	30.00	30.00
Soybean meal (46.5% CP)	9.20	9.94	10.68	9.20	9.94	10.68
Corn oil	2.00	4.00	6.00	-	-	-
Choice white grease	-	-	-	2.00	4.00	6.00
Monocalcium phosphate	1.28	1.26	1.25	1.28	1.26	1.25
Lysine sulphate (54.6%)	0.31	0.32	0.32	0.31	0.32	0.32
Salt	0.36	0.37	0.37	0.36	0.37	0.37
Vitamin and trace mineral premix[1]	0.12	0.12	0.12	0.12	0.12	0.12
Analyzed composition						
DM, %	89.2	89.3	89.5	89.2	89.3	89.6
GE, Mcal/kg	4.12	4.20	4.26	4.09	4.20	4.28
Acid hydrolyzed ether extract, %	7.62	9.04	9.99	7.33	9.21	10.34
Diet IV[2], g/100g	119.6	119.0	117.0	105.5	92.5	91.1
IVP[3]	91.1	107.6	116.8	77.3	85.1	94.2
Fatty acid[4], %						
C16:0	16.2	17.1	16.8	18.9	23.2	21.2
C18:0	2.7	2.5	2.8	6.3	8.4	9.1
C18:1	25.6	24.7	25.4	28.3	30.0	32.5
C18:2	53.6	53.7	51.4	44.2	36.5	33.8
C18:3	1.7	1.7	1.6	1.5	1.0	1.7
Other	0.4	0.4	2.0	0.8	0.9	2.2
Omega-3 to omega-6 ratio	0.032	0.032	0.031	0.034	0.027	0.050
Calculated composition						
ME, Mcal/kg	3.36	3.45	3.54	3.36	3.45	3.54

[1]Provides 2,937 IU vitamin A, 734 IU vitamin D, 14 IU vitamin E, 1.5 mg menadione, 14.7 mg vitamin B_{12}, 2.2 mg riboflavin, 18.4 mg niacin, 11.0 mg pantothenic acid, 257.2 mg Co (cobalt carbonate), 6.6 g Cu (copper sulfate), 147.0 mg I (calcium iodine), 73.4 g iron Fe (iron sulfate), 20.3 g Mn (manganese sulfate), 198.4 mg Se (sodium selenite), 73.4 g Zn (zinc sulfate) per kilogram of diet.

[2]Iodine value calculated from fatty acid composition: (IV) = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate percentage concentration (AOCS, 1998).

[3]Iodine value product (IVP) = [IV of dietary lipids × ether extract (%)] × 0.10 (Christensen, 1962; Madsen et al., 1992).

[4]Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3).

Table 2.4. Ingredient and nutrient composition (as-fed basis) of the experimental diets, d 63 to 134

	Corn oil			Choice white grease		
Item	2%	4%	6%	2%	4%	6%
Ingredient, %						
Corn[1]	68.94	68.21	63.47	68.94	68.21	63.47
Dried distillers grains with solubles	15.00	15.00	15.00	15.00	15.00	15.00
Soybean meal (46.5% CP)	12.16	12.90	13.64	12.16	12.90	13.64
Corn oil	2.00	4.00	6.00	-	-	-
Choice white grease	-	-	-	2.00	4.00	6.00
Monocalcium phosphate	1.01	1.00	0.99	1.01	1.00	0.99
Lysine sulphate (54.6%)	0.30	0.30	0.31	0.30	0.30	0.31
L-threonine (98.5%)	0.01	0.02	0.02	0.01	0.02	0.02
Salt	0.43	0.43	0.43	0.43	0.43	0.43
Vitamin and trace mineral premix[2]	0.12	0.12	0.12	0.12	0.12	0.12
Optiphos 1000[3]	0.01	0.01	0.01	0.01	0.01	0.01
Analyzed composition						
DM, %	88.9	89.1	89.5	89.0	89.3	89.6
GE, Mcal/kg	4.04	4.14	4.27	4.03	4.17	4.26
Acid hydrolyzed ether extract, %	6.04	7.37	8.53	6.22	7.83	9.11
Diet IV[4], g/100g	118.7	119.5	120.6	103.3	94.8	87.3
IVP[5]	71.7	88.1	102.9	64.2	74.2	79.5
Fatty acid[6], %						
C16:0	16.2	15.6	15.4	19.6	20.8	23.2
C18:0	3.1	2.6	2.4	6.6	8.4	9.5
C18:1	25.7	25.8	25.2	29.0	32.2	33.5
C18:2	53.0	54.5	54.4	42.3	35.9	30.6
C18:3	1.7	0.8	1.6	1.6	1.4	1.5
Other	0.2	0.7	1.1	0.8	1.3	1.6
Omega-3 to omega-6 ratio	0.032	0.015	0.029	0.038	0.039	0.049
Calculated composition						
ME, Mcal/kg	3.39	3.48	3.57	3.39	3.48	3.57

[1]Titanium dioxide was included at 0.40% as an indigestible marker in substitution of corn from d 95 to 105.

[2]Provides 2,937 IU vitamin A, 734 IU vitamin D, 14 IU vitamin E, 1.5 mg menadione, 14.7 mg vitamin B_{12}, 2.2 mg riboflavin, 18.4 mg niacin, 11.0 mg pantothenic acid, 257.2 mg Co (cobalt carbonate), 6.6 g Cu (copper sulfate), 147.0 mg I (calcium iodine), 73.4 g iron Fe (iron sulfate), 20.3 g Mn (manganese sulfate), 198.4 mg Se (sodium selenite), 73.4 g Zn (zinc sulfate) per kilogram of diet.

[3]Huvepharma, Inc., Peachtree City, GA.

[4]Iodine value calculated from fatty acid composition: (IV) = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate percentage concentration (AOCS, 1998).

[5]Iodine value product (IVP) = [IV of dietary lipids × ether extract (%)] × 0.10 (Christensen, 1962; Madsen et al., 1992).

[6]Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3).

Table 2.5. Analyzed composition of dietary fat sources[1]

Item	Corn oil[2]	Choice white grease[3]
Free fatty acids, %	11.60	1.96
MIU[4], %	0.55	0.57
Moisture and volatile matter, %	0.02	0.14
Insoluble impurities, %	0.14	0.03
Unsaponifiable matter, %	0.39	0.40
Initial peroxide value, meq/kg	0.20	8.20
Fatty acid[5], %		
C14:0	0.1	1.3
C16:0	11.3	23.0
C16:1	0.3	2.0
C17:0	0.3	0.3
C18:0	8.1	13.1
C18:1	21.9	41.3
C18:2	50.5	15.7
C18:3	6.3	0.6
C20:0	0.3	0.3
C20:1	0.1	0.7
C20:2	nd[7]	0.8
C20:4	nd	0.4
C22:0	0.3	nd
Other fatty acids	0.5	0.8
Iodine value, g/100 g[6]	123.2	66.8

[1]Analysis via Barrow-Agee Laboratories (Memphis, TN).

[2]Distiller's corn oil sourced via POET (Growie, IA).

[3]Sourced via Sanimax (South St. Paul, MN).

[4]Moisture, impurities, and unsaponifiables.

[5]Myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), margaric acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), gadoleic acid (C20:1), eicosadienoic acid (C20:2), arachidonic acid (C20:4), behenic acid (C22:0).

[6]Iodine value calculated from fatty acid composition: (IV) = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate percentage concentration (AOCS, 1998).

[7]Non-detectable.

Table 2.6. Effects of dietary fat source (FS) and level (FL) on overall (d 0 to 134) growth performance and feed efficiency

	Treatment						Sex			*P*-value			
	Corn oil			Choice white grease									
Item	2%	4%	6%	2%	4%	6%	Barrow	Gilt	SEM	Sex	FS	FL	FS × FL
No. of pens[1]	10	10	10	10	10	10	30	30					
ADG, kg	0.902	0.917	0.921	0.902	0.903	0.926	0.939	0.883	0.008	<0.001	0.907	0.266	0.558
ADFI, kg	2.601	2.574	2.472	2.582	2.493	2.463	2.632	2.430	0.032	<0.001	0.325	0.028	0.682
G:F	0.348	0.357	0.371	0.350	0.363	0.377	0.357	0.364	0.003	0.029	0.107	<0.001	0.876
BW d 0	31.9	32.2	31.6	32.2	32.0	32.3	32.3	31.7	0.4	0.174	0.572	0.954	0.667
Average market BW, kg[2, 3]	139.5	140.6	140.6	140.0	139.8	140.4	140.7	139.6	0.7	0.029	0.749	0.513	0.560
Pig days[4]/number of head sold	119	119	119	120	119	116	116	122	2	<0.001	0.407	0.417	0.286

[1]Pens had ~20 pigs/pen.

[2]Average of live pen BW taken on d 105, 117, and 134.

[3]Difference among marketing pulls was evident for average market BW (d 105 = 137.8, d 117 = 138.8, d 134 = 143.5 kg; $P < 0.001$).

[4]Pig days was calculated as the number of pigs in pen per d.

Table 2.7. Effects of dietary fat source (FS) and level (FL) on dietary fatty acid intake[1], d 0 to 134

	Treatment						Sex			*P*-value			
	Corn oil			Choice white grease									
Item	2%	4%	6%	2%	4%	6%	Barrow	Gilt	SEM	Sex	FS	FL	FS × FL
No. of pens[2]	10	10	10	10	10	10	30	30					
C16:0[3], g/d	30.4	36.5	39.2	34.7	48.6	53.7	42.1	38.9	0.6	<0.001	<0.001	<0.001	<0.001
C18:0[4], g/d	5.3	5.5	6.7	11.4	17.9	23.0	12.1	11.2	0.2	<0.001	<0.001	<0.001	<0.001
C18:1[5], g/d	48.1	55.6	61.3	50.8	67.8	81.9	63.4	58.5	0.9	<0.001	<0.001	<0.001	<0.001
C18:2[6], g/d	101.1	118.0	126.1	77.2	79.9	82.4	101.4	93.6	1.4	<0.001	<0.001	<0.001	<0.001
C18:3[7], g/d	3.2	3.3	4.1	2.7	2.4	3.5	3.3	3.1	0.1	<0.001	<0.001	<0.001	0.012

[1]Fatty acid intake (g/d) = ADFI (g/d) × dietary fatty acid (%) × dietary acid hydrolyzed ether extract (%; Kellner et al., 2014).
[2]Pens had ~20 pigs/pen.
[3]Palmitic acid (C16:0).
[4]Stearic acid (C18:0).
[5]Oleic acid (C18:1).
[6]Linoleic acid (C18:2).
[7]Linolenic acid (C18:3).

Table 2.8. Effects of dietary fat source (FS) and level on ME intake and caloric efficiency, d 0 to 134

	Treatment						Sex			*P*-value			
	Corn oil			Choice white grease									
Item	2%	4%	6%	2%	4%	6%	Barrow	Gilt	SEM	Sex	FS	FL	FS × FL
No. of pens[1]	10	10	10	10	10	10	30	30					
ME intake, Mcal/d	8.71	8.91	8.77	8.65	8.63	8.74	9.08	8.39	0.11	<0.001	0.323	0.835	0.677
ME intake:BW gain, Mcal/kg	9.66	9.71	9.58	9.58	9.54	9.44	9.67	9.50	0.07	0.034	0.109	0.428	0.888
ME intake:carcass gain, Mcal/kg	12.93	12.92	12.82	12.82	12.70	12.53	12.92	12.66	0.10	0.024	0.074	0.350	0.836

[1]Pens had ~20 pigs/pen.

Table 2.9. Effects of dietary fat source (FS) and level (FL) on apparent total tract digestibility (ATTD) of dietary DM, GE, and acid hydrolysis ether extract (AEE)[1] and true total tract digestibility (TTTD)[2] of dietary AEE on d 39 and d 104

	Treatment						Sex			*P*-value			
	Corn oil			Choice white grease									
Item	2%	4%	6%	2%	4%	6%	Barrow	Gilt	SEM	Sex	FS	FL	FS × FL
No. of pens[3]	10	10	10	10	10	10	30	30					
d 39													
ATTD of DM, %	77.4	82.0	83.7	78.3	82.0	80.5	80.3	81.0	0.6	0.041	0.042	<0.001	<0.001
ATTD of GE, %	78.6	82.7	84.2	78.8	82.5	81.1	81.0	81.6	0.3	0.051	0.005	<0.001	<0.001
ATTD of AEE, %	66.2	74.9	79.2	65.2	75.4	75.7	72.6	73.0	0.9	0.490	0.017	<0.001	0.013
TTTD of AEE, %	95.0	94.1	93.4	91.6	92.8	92.9	93.2	93.4	0.8	0.861	0.066	0.955	0.404
d 104													
ATTD of DM, %	77.7	81.5	83.5	76.5	81.6	80.7	79.8	80.3	0.7	0.292	0.049	<0.001	0.010
ATTD of GE, %	78.5	82.1	84.2	77.1	82.4	81.6	80.7	80.8	1.0	0.953	0.049	<0.001	0.005
ATTD of AEE, %	54.0	66.1	71.3	54.9	68.5	68.9	64.1	63.7	1.0	0.484	0.641	<0.001	0.007
TTTD of AEE, %	98.5	99.0	99.0	98.4	99.1	98.6	98.9	98.8	0.1	0.608	0.402	<0.001	0.222

[1]Apparent total tract digestibility (ATTD; %) of either AEE, DM, or GE was calculated as 100 – {100 × [concentration (g) of TiO_2 in diet × concentration of (g) of AEE, DM, or GE in feces]/[concentration (g) of TiO_2 in feces × concentration of AEE, DM, or GE in diet]}; (Oresanya et al. 2007).

[2]Calculated via correcting ATTD of AEE for endogenous fat losses at 20 g of AEE/kg of DM intake.

[3]Pens had ~20 pigs/pen.

Table 2.10. Effects of dietary fat source (FS) and level (FL) on carcass characteristics pooled across all marketing pulls (d 105, 117, and 134)[1]

	Treatment						Sex			*P*-value			
	Corn oil			Choice white grease									
Item	2%	4%	6%	2%	4%	6%	Barrow	Gilt	SEM	Sex	FS	FL	FS × FL
No. of pens[2]	10	10	10	10	10	10	30	30					
HCW[3], kg	104.2	105.7	105.1	104.7	105.0	105.7	105.4	104.7	0.6	0.190	0.791	0.153	0.471
Yield, %	74.7	75.1	74.8	74.7	75.1	75.3	74.9	75.0	0.1	0.299	0.276	0.069	0.330
Backfat[4], cm	1.99	2.02	2.05	1.94	1.98	2.00	2.02	1.89	0.04	<0.001	0.180	0.287	0.998
Loin depth[5], cm	7.20	7.18	7.15	7.18	7.17	7.15	7.11	7.24	0.05	0.002	0.826	0.670	0.982
Percent lean[6], %	55.4	55.3	55.2	55.5	55.4	55.3	54.9	55.7	0.1	<0.001	0.418	0.274	0.989

[1]No pull × treatment interaction was evident for any carcass measurement ($P \geq 0.489$).

[2]Pens had ~20 pigs/pen.

[3]Difference among pulls was evident for HCW (d 105 = 104.7, d 117 = 103.7, d 134 = 106.0 kg; $P < 0.001$).

[4]Difference among pulls was evident for backfat (d 105 = 1.88, d 117 = 1.97, d 134 = 2.17 cm; $P < 0.001$).

[5]Difference among pulls was evident for loin depth (d 105 = 7.06, d 117 = 7.20, d 134 = 7.25 cm; $P < 0.001$).

[6]Difference among pulls was evident for percent lean (d 105 = 55.4, d 117 = 55.4, d 134 = 55.0%; $P < 0.001$).

Table 2.11. Effects of dietary fat source (FS) and level (FL) on fatty acid profile and calculated carcass iodine value (IV)[1] of carcass belly fat pooled across all marketing pulls (d 105, 117, and 134)[2]

	Treatment						Sex			*P*-value			
	Corn oil			Choice white grease									
Item	2%	4%	6%	2%	4%	6%	Barrow	Gilt	SEM	Sex	FS	FL	FS × FL
No. of pens[3]	10	10	10	10	10	10	30	30					
Fatty acid[4], %													
C10:0	0.08	0.05	0.03	0.07	0.09	0.04	0.06	0.06	0.01	0.867	0.351	0.028	0.379
C12:0	0.04	0.03	0.02	0.05	0.03	0.03	0.03	0.03	0.01	0.307	0.078	<0.001	0.726
C13:0	0.11	0.13	0.12	0.13	0.15	0.12	0.12	0.13	0.01	0.712	0.074	0.222	0.627
C14:0	1.41	1.36	1.17	1.51	1.46	1.64	1.47	1.38	0.06	0.222	0.001	0.720	0.039
C16:0	22.36	21.46	19.67	23.62	22.76	22.99	22.67	21.62	0.14	<0.001	<0.001	<0.001	<0.001
C16:1	2.12	1.88	1.47	2.33	2.29	2.16	2.20	1.89	0.03	<0.001	<0.001	<0.001	<0.001
C17:0	0.38	0.35	0.31	0.41	0.38	0.37	0.37	0.36	0.01	0.346	<0.001	<0.001	0.310
C17:1	0.23	0.19	0.13	0.29	0.25	0.24	0.24	0.21	0.01	<0.001	<0.001	<0.001	0.019
C18:0	9.75	9.33	9.26	10.42	9.82	10.28	9.62	9.69	0.11	0.561	<0.001	<0.001	<0.001
C18:1	37.20	35.74	33.50	39.66	40.39	39.95	38.11	37.37	0.42	0.099	<0.001	0.007	0.002
C18:2	23.33	26.45	31.89	18.53	19.29	19.09	22.12	24.07	0.39	<0.001	<0.001	<0.001	<0.001
C18:3	0.74	0.80	0.91	0.64	0.67	0.69	0.71	0.77	0.01	<0.001	<0.001	<0.001	0.005
C20:0	0.15	0.15	0.12	0.14	0.12	0.12	0.14	0.13	0.01	0.661	0.258	0.021	0.287
C20:1	0.65	0.64	0.59	0.75	0.82	0.87	0.73	0.70	0.01	0.012	<0.001	0.139	<0.001
C20:2	0.91	1.02	1.17	0.93	0.87	0.89	0.92	1.01	0.04	0.461	0.005	0.172	0.046
C20:3	0.35	0.33	0.33	0.32	0.34	0.35	0.32	0.35	0.01	0.060	0.962	0.987	0.483
C20:4	0.05	0.03	0.06	0.05	0.08	0.04	0.04	0.06	0.01	0.325	0.586	0.933	0.179
C20:5	0.05	0.04	0.06	0.05	0.06	0.05	0.05	0.05	0.01	0.596	0.665	0.388	0.145
C22:1	0.08	0.09	0.09	0.08	0.10	0.11	0.08	0.10	0.01	0.008	0.306	0.192	0.269
Other SFA[5]	nd[9]	nd	nd	nd	0.01	0.01	0.01	nd	-	-	-	-	-
Other UFA[6]	nd	nd	nd	nd	Nd	nd	nd	nd	-	-	-	-	-
IV[7], g/100g	76.9	81.0	88.3	70.7	72.8	72.0	75.7	78.3	0.5	<0.001	<0.001	<0.001	<0.001
n-3/n-6 ratio[8]	0.047	0.043	0.039	0.052	0.053	0.055	0.050	0.050	0.001	0.653	<0.001	0.026	<0.001

[1]Iodine value was calculated by: [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate percentage concentration (AOCS, 1998).

[2]No pull × treatment interaction was evident for carcass fatty acid profile or carcass IV ($P \geq 0.171$).

[3]Pens had ~20 pigs/pen.

[4]Capric acid (C10:0), lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), margaric acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), gadoleic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), timnodonic acid (C20:5), erucic acid (C22:1).

[5]Saturated fatty acids.

[6]Unsaturated fatty acids.

[7]No difference was evident among marketing pulls for carcass IV (d 105 = 77.2, d 117 = 77.2, d 134 = 76.7 g/ 100 g; $P = 0.899$).

[8]Omega-3 fatty acid to Omega-6 fatty acid ratio.

[9]Non-dectectable.

Table 2.12. Comparison of iodine value product (IVP)[1], dietary fatty acid concentration[2], and daily fatty acid intake[3] as predictors of pork carcass iodine value (IV)[4] pooled across 3 marketing pulls (d 105, 117, and 134)

Predictor	IV (g/100g) =	R^2	*P*-value	Root MSE[5]
IVP	42.99 + (0.373 × IVP)	0.85	0.008	2.87
C16:0, %	84.70 – (4.822 × dietary 16:0%)	0.08	0.596	7.22
C16:0 intake	86.55 – [0.237 × 16:0 intake/d (g)]	0.10	0.547	7.14
C18:0, %	81.80 – (4.699 × dietary 18:0%)	0.29	0.275	6.35
C18:0 intake	83.89 – [0.596 × 18:0 intake/d (g)]	0.43	0.160	5.70
C18:1, %	82.17 – (2.160 × dietary 18:1%)	0.03	0.736	7.40
C18:1 intake	83.62 – [0.110 × 18:1 intake/d (g)]	0.04	0.698	7.36
C18:2, %	49.94 + (7.000 × dietary 18:2%)	0.95	<0.001	1.69
C18:2, % and ME intake	205.76 + (8.48 × dietary 18:2%) – [(18.50 × ME intake/d (Mcal/kg)]	0.98	0.002	1.12
C18:2 intake	46.74 + [0.310 × 18:2 intake/d (g)]	0.94	0.002	1.92
C18:3, %	51.33 + (202.53 × dietary 18:3%)	0.57	0.084	4.94
C18:3 intake	52.13 + [8.051 × 18:3 intake/d (g)]	0.27	0.289	6.41

[1]Iodine value product (IVP) = [IV of dietary lipids × ether extract (%)] × 0.10 (Christensen, 1962; Madsen et al., 1992).

[2]Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3).

[3]Fatty acid intake (g/d) = ADFI (g/d) × dietary fatty acid (%) × dietary acid hydrolyzed ether extract (%; Kellner et al., 2015).

[4]Iodine value was calculated by: [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate percentage concentration (AOCS, 1998).

[5]MSE = mean squared error.

CHAPTER III

DOES HEAT STRESS ALTER THE PIG'S RESPONSE TO DIETARY FAT?

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T. A. Kellner,* L. H. Baumgard,* K. J. Prusa,† N. K. Gabler,* and J. F. Patience*

*Department of Animal Science, Iowa State University, Ames, 50011, USA; and †Department of Food Science and Human Nutrition, Iowa State University, Ames, 50011, USA

Abstract

Heat stress (HS) results in major losses to the pork industry via reduced growth performance and possibly carcass fat quality. The experimental objective was to measure the effects of HS on the pig's response to dietary fat in terms of lipid digestion, metabolism, and deposition over a 35 d finishing period. A total of 96 PIC 337 × C22/C29 (PIC, Inc., Hendersonville, TN) barrows (initial BW of 100.4 ± 1.2 kg) were allotted randomly to 1 of 9 treatments arranged as a 3 × 3 factorial: [TN (thermonetural: constant 24°C; ad libitum access to feed), PFTN (pair-fed thermoneutral: constant 24°C; limit-fed based on previous HS daily feed intake), or HS (cyclical 28°C nighttime, 33°C d 0 to 7, 33.5°C d 7 to 14, 34°C d 14 to 21, 34.5°C d 21 to 28, 35°C d 28 to 35 daytime; ab libitum access to feed)] and diet [a corn-soybean meal based diet with 0% added fat (CNTR), 3% added tallow (TAL; iodine value (IV) = 41.8), or 3% added corn oil (CO; IV = 123.0)]. No interactions between environment and diet were evident for any major response criteria ($P \geq 0.063$). Rectal temperature increased due to HS (HS = 39.0,

TN = 38.1, PFTN = 38.2°C; $P < 0.001$). Heat stress decreased ADFI (27.8%; $P < 0.001$), ADG (HS = 0.72, TN = 1.03, PFTN = 0.78 kg/d; $P < 0.001$), and G:F (HS = 0.290, TN = 0.301, PFTN = 0.319; $P = 0.006$). Heat stress barrows required 1.2 Mcal of ME intake more per kg of BW gain than PFTN ($P < 0.001$). Heat stress tended to result in the lowest ATTD of AEE (HS = 59.0, TN = 60.2, PFTN = 61.4%, $P = 0.055$). True total tract digestibility of AEE of CO-based diets (99.3%) was greater than that of CNTR (97.3%) and TAL-based diets (96.3%; $P = 0.012$). Environment had no impact on TTTD of AEE ($P = 0.118$). Environment had no impact on jowl IV at market (HS = 69.2, TN = 69.3, PFTN = 69.8 g/100 g; $P = 0.624$). Jowl IV at market increased with increasing degree of unsaturation of the dietary fat (CNTR = 68.5, TAL = 68.2, CO = 71.5 g/100 g; $P < 0.001$). Heat stress decreased mRNA abundance of *ATGL* and *HSL* ($P \leq 0.041$). HS and CO increased mRNA abundance of *SCD* ($P \leq 0.047$), and CO increased abundance of *FASN* ($P = 0.011$). In conclusion, HS does not alter the pig's response to dietary fat. However, HS leads to reduced ADG, ADFI, G:F, caloric efficiency, and a suppression of mRNA abundance of genes involved in the lipolytic cascade, which resulted in a phenotype that was fatter than PFTN.

Introduction

Heat stress (**HS**) affects a plethora of swine production variables (Baumgard et al., 2012); its negative impact on ADG has been known for over 110 yrs (Grisdale, 1904; Heitman et al., 1958). Despite improvements in barn design, genetics, management, and nutrition, HS remains one of the most costly issues for American pork producers (St-Pierre et al., 2003; Renaudeau et al., 2012).

To reduce heat stress's negative impact on energy intake (Hao et al., 2014; Pearce et al., 2014), producers formulate diets utilizing ingredients that are energy dense and low in heat increment (Forbes and Swift, 1944; Stahly et al., 1981). Because dietary fat and oils are energy dense and have a low heat increment, (NRC, 2012; Kerr et al., 2015), their use increases in the hotter months of the year. Adding dietary fat has been shown to reduce the negative effects of HS on ADG (Stahly et al., 1981; Spencer et al., 2005). What is unknown is whether high ambient temperature affects the pig's utilization of fat, and if a fat source that is more unsaturated will be more effective at alleviating the negative effects of HS.

A review by Baumgard and Rhoads (2013) concluded that pigs that experience HS deposit more lipid than predicted based on their energy consumption. It is also known that the composition of dietary fat will be highly reflective of pork fat composition (Ellis and Isbell, 1926; Kellner et al., 2014). This creates a scenario where high fat diets are employed to alleviate HS and HS pigs deposit even greater amounts of fat than expected, increasing the risk of carcass fat quality issues when HS occurs (Spencer et al., 2005; White et al., 2008).

The experimental objective was to determine if HS would impact the pig's response to a more saturated or a less saturated dietary fat source in terms of growth performance, caloric efficiency, lipid metabolism, carcass quality, and carcass iodine value (**IV**).

Materials and methods

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research, and were approved by the Iowa State University Institutional Animal Care and Use Committee (#1-14-7703-S).

Animals, Housing, and Experimental Design

A total of 96 PIC 337 × C22/C29 (PIC, Inc., Hendersonville, TN) barrows, with an average initial BW of 100.4 ± 1.2 kg were allotted by BW and pre-experiment ADG to 1 of 9 treatments arranged as a 3 × 3 factorial. The first factor was environmental treatment: thermoneutral (**TN**; ad libitum access to feed), pair-fed thermoneutral (**PFTN**; limit-fed based on HS feed intake on the previous day), or HS (ab libitum access to feed). The second factor was diet: a corn-soybean meal based diet with 0% added fat (**CNTR**), CNTR with 3% added tallow (**TAL**; IV = 41.8), or CNTR with 3% added corn oil (**CO**; IV = 123.0). There were 2 sequential replications of 48 barrows each.

Pigs were housed in 2 identical rooms where temperature was controlled (Figure 3.1), but humidity, while similar between the 2 rooms, was not regulated (Figure 3.2). Each room contained 24 individual pens. Each pen provided 1.25 m^2 of floor space, a nipple drinker, a stainless steel feeder, and had mesh metal flooring. Pigs were given ad libitum access to water throughout the experiment.

The control room housed TN and PFTN barrows and was maintained within the thermoneutral temperature zone for pigs of this age (24°C; Comberg et al., 1972; Renaudeau et al., 2012). The HS room housed HS barrows and was heated in a diurnal pattern (Figure 3.1) at 28°C from 2000 h to 800 h and at 33°C d 0 to 7, 33.5°C d 7 to 14, 34°C d 14 to 21, 34.5°C d 21 to 28, 35°C d 28 to 35 from 800 h to 2000 h. The temperature of the HS room was set greater than estimated upper critical temperature point from 800 h to 2000 h and set slightly less than the estimated upper critical temperature point from 2000 h to 800 h based on multiple studies complied by Renaudeau et al. (2012). Additionally, the upper temperature of the HS room was increased 0.5°C every 7 d to minimize acclimation to the environmental conditions during the 35

d experiment. Temperature and humidity in both rooms were recorded every 30 min using a data logger (Lascar EL-USB-2-LCD, Lascar Electronics, Erie, PA).

Diets and Feeding

All experimental diets (Table 3.1) were formulated on a constant ME to standardized ileal digestible lysine ratio and met or exceeded all nutrient requirements for pigs of this size (NRC, 2012). Diets contained 0.40% titanium dioxide as an indigestible marker to determine the apparent total tract digestibility (**ATTD**) of acid hydrolyzed ether extract (**AEE**), DM, and GE. All experimental diets were offered to the pigs in mash form. Dietary fat sources were selected to provide a diverse range of unsaturation, while keeping in mind choices relevant to current production practices. Representative feed samples were collected at the time of mixing and stored at -20°C for later analysis. Prior to the initiation of the study, the pigs were fed a common diet, similar to the experimental CNTR diet.

Sample Collection

Pigs were weighed individually on d 0, 7, 14, 21, and 35. Feeders in the TN pens were weighed on d 0, 7, 14, 21, and 35. Feeders in the HS room were weighed daily to determine daily feed intake for the next d PFTN feed allotment. If any feed was remaining in the feeders of PFTN barrows at 800 h, it was measured and discarded before the next daily allotment of feed was added. These measurements allowed for the determination of ADG, ADFI, and G:F. Fecal grab samples were collected fresh from each pig on d 16 to 18, and immediately stored at -20°C for later analysis.

Rectal temperature was measured daily with a dual-scale digital thermometer at 1100 h (VetOne; MWI Veterinary Supply, Boise, ID). Daily respiration rate was determined by counting flank movements at 1200 h. Both measurements were taken in duplicate and condensed into daily averages if numerical differences occurred.

Subcutaneous fat samples from the jowl were collected on d 7 and 21 by biopsy, while under local anesthesia. The skin was removed from each 10 g lipid sample. Once the skin was removed a ~200 mg cross section was taken and placed into a 2.5 mL ribonuclease-free microcenterfuge tube (FisherBrand; Fisher Science, Hanover Park, IL) with 2 mL of TRIzol reagent (Invitrogen, Carlsbad, CA). The remaining lipid sample was inserted into a 7.62 by 12.70 cm plastic bag (FisherBrand; Fisher Science, Hanover Park, IL) and snap-frozen using liquid nitrogen. These samples were immediately placed on dry ice and then stored at -80°C for later analysis.

On d 35, pigs were marketed at the JBS professing plant in Marshalltown, IA, where HCW, loin depth, and back fat thickness were measured. Following carcass chilling, a 100 g sample of fat from the right jowl of each carcass was collected, vacuum packaged, and stored at -20°C until analyzed. The loin from the right side of each carcass was measured for pH using a Hanna HI925 meter with an FC200 hard glass electrode (Hanna Instruments, Woonsocket, RI), for loin color score (Japanese color bar 1 to 6, with 1 = extremely light and 6 = extremely dark; Sullivan et al., 2007), and for loin marbling score according to National Pork Board Standards (NPPC, 2000). The right side of the belly from each carcass was collected and measured for weight, temperature, and thickness. Belly thickness was measured in 2 locations in the center of the belly for middle thickness and at the center of the scribe edge of the belly for edge thickness. A belly firmness test was conducted using a durometer (model 1600-OOO-S; Electromatic

Equipment Co., Inc., Cedarhurst, NY) which measured compression of the belly (1 to 100, with 1 = least firm and 100 = firmest; Semen et al., 2013; Kellner et al., 2015). A subjective belly firmness test was conducted by assigning a visual score (1 to 3, 1 = firmest and 3 = least firm) based on the degree of flop of the belly (Kellner et al., 2014). Objective color measures were obtained using a Minolta Chromameter CR 310 (Minolta Corp., Ramsey, NJ) equipped with a 50 mm orifice calibrated against a white tile. Objective color and durometer measures were taken in the middle of the belly with skin removed 3 cm from the proximal edge. Temperature of each belly analyzed was recorded with a thermometer (model 7937; Fisher Science, Hanover Park, IL). No treatment differences among belly temperatures were evident (2.5 ± 0.7°C; $P = 0.580$).

Analytical Methods

Fatty acids were extracted from adipose tissue and feed samples by a 1-step direct transesterification procedure (Lepage and Roy, 1986). The fatty acid profile was then determined by gas chromatography using a model 3900 gas chromatograph fitted with a CP 8400 automatic injector (Varian Inc., Walnut Creek, CA) and a 60 m capillary column (0.25 mm diameter; model DB-23; Agilent Technologies, Santa Clara, CA). Helium was utilized as a carrier gas at 0.5 mL/mm (1:50 split ratio). Oven temperature started at 50°C and increased to 235°C across a 26 min period. The injector and detector were maintained at 250°C. Identification of fatty acid peaks was performed by comparison with purified fatty acid samples obtained from Sigma-Aldrich, Co. (St. Louis, MO).

Prior to analysis, fecal and feed samples were homogenized and then finely ground through a 1 mm screen in a Retsch grinder (model ZM1; Retsch Inc., Newtown, PA). Acid hydrolyzed ether extract (method 2003.06, AOAC International, 2007) was analyzed using a

SoxCap SC 247 hydrolyzer and a Soxtec 255 semiautomatic extractor (FOSS North America, Eden Prairie, MN). Dry matter was determined according to modified methods (930.15, AOAC International, 2007) by drying samples in an oven at 105°C to a constant weight. Gross energy was determined using a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). Benzoic acid (6.318 Mcal/kg; Parr Instruments) was used as the standard for calibration (determined GE: 6.320 ± 0.006 Mcal/kg). Titanium dioxide was determined by spectrophotometer (synergy 4; BioTek Instruments Inc., Winooski, VT) according to the method of Leone (1973). All chemical analyses were performed in duplicate and repeated when intra-duplicate CV was greater than 1%.

Adipose tissue stored in TRIzol was homogenized using a Clean PowerGen 700D homogenizer (Fisher Science, Hanover Park, IL). Total RNA was then isolated from adipose tissue using TRIzol reagent following the manufacturer's protocol with the modification of repeating the RNA pellet wash step 3 times to reduce 230 nm contamination. Isolated RNA was then utilized for cDNA synthesis employing the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). Abundance differences of mRNA were determined using quantitative PCR (BioRad iCycler; Hercules, CA) on 12 genes. Expression normalization across samples within tissue was performed by calculating a delta cycle threshold (**Ct**) value for each sample using *RPL32*, as transcript abundance proved to be similar among treatments ($P < 0.05$).

Calculations

According to the equation of Oresanya et al. (2007), ATTD, % of AEE, DM, and GE was calculated as 100 – {100 × [concentration (g) of TiO_2 in diet × concentration of (g) of AEE, DM, or GE in feces]/[concentration (g) of TiO_2 in feces × concentration of AEE, DM, or GE in diet]}.

True total tract digestibility (**TTTD**; %) of AEE was calculated by correcting ATTD of AEE for endogenous fat losses at 20 g of AEE/kg of DM intake (Acosta Camargo et al., 2015).

Delta delta Ct (**ΔΔCt**) values were calculated from delta Ct values using a reference sample. Fold differences among treatments were calculated using the following equation $2^{|\Delta\Delta Ct(\text{treatment A}) - \Delta\Delta Ct(\text{treatment B})|}$. The fold difference among treatments are expressed where a positive value indicates an increase in transcript abundance and negative value indicates a decrease.

Iodine value was calculated from the fatty acid profile using the following equation: IV = (C16:1 × 0.95) + (C18:1 × 0.86) + (C18:2 × 1.732) + (C18:3 × 2.616) + (C20:1 × 0.785) + (C22:1 × 0.723); (AOCS, 1998).

Statistical Analysis

Analysis of the 9 treatments arranged as a 3 × 3 factorial, the main effects of environment (TN vs. PTFN vs. HS) and dietary fat (CNTR vs. CO vs. TAL), and their interactions (**E × DF**) were analyzed using PROC MIXED (SAS 9.4; SAS Inst. Inc., Cary, NC) with replicate as a random effect. Pig was the experimental unit. For each variable, normal distribution of residuals was tested using PROC UNIVARIATE.

Non-detectable fatty acid values were treated in all statistical analyses as 0. All *P*-values less than 0.05 were considered significant and *P*-values between 0.05 and 0.10 were considered trends.

Results

Environment and Dietary Fat Effects on Rectal Temperature and Respiration Rate

As expected, during the 35 d experimental period HS pigs had an increased rectal temperature and greater than twice the respiration rate of TN and PFTN pigs ($P < 0.001$; Table 3.2). Dietary fat had no impact on either rectal temperature or respiration rate ($P \geq 0.203$). There was no E × DF interaction evident for rectal temperature or respiration rate, which indicates that HS pigs sustained a heat load indicative of marked HS and that dietary fat did not increase or decrease the degree of HS ($P \geq 0.192$).

Environment and Dietary Fat Effects on Growth Performance, Feed Intake, and Feed Efficiency

There were no E × DF interactions for ADG, ADFI, or gain to feed ratio ($P \geq 0.157$; Table 3.2). As expected, the ADG of TN pigs was greater than PFTN and HS pigs ($P < 0.001$). Dietary fat had no impact on ADG ($P \geq 0.413$; Table 3.2). As expected, the ADFI of TN pigs was greater than HS pigs, and by design the ADFI of HS and PFTN pigs were not different ($P < 0.001$; Table 3.2). Overall, PFTN barrows converted gain from feed with greater efficiency than HS barrows ($P < 0.001$; Table 3.2). Overall, a CO-based diet tended to increase gain to feed ratio with TAL as the intermediate and CNTR as the least efficient ($P = 0.073$; Table 3.2). Part of the difference between the fat sources could be due to slight differences in their available energy content.

Environment and Dietary Fat Effects on Energy Intake and Caloric Efficiency

No E × DF interactions were evident for energy intake or caloric efficiency ($P \geq 0.477$; Table 3.3). By design, ME intake of HS and PFTN pigs were similar and both were less than TN pigs ($P < 0.001$). Barrows in the HS environment required more Mcal of ME to deposit 1 kg of BW or 1 kg of carcass weight than PFTN ($P \leq 0.021$). There was a tendency for barrows fed a TAL-based diet to consume less energy/d ($P = 0.090$), but there was no impact of dietary fat on caloric efficiency ($P \geq 0.654$).

Environment and Dietary Fat Effects on Digestibility of Dry Matter, Energy, and Lipids

No E × DF interactions were evident for digestibility of DM, GE, or AEE ($P \geq 0.253$; Table 3.4). No differences were evident among environment or dietary fat treatments for ATTD of DM ($P \leq 0.223$). The ATTD of GE was decreased in TN pigs when compared with PFTN and HS pigs ($P = 0.008$). Barrows in the HS environment compared to a TN environment tended to have decreased ATTD of AEE ($P = 0.055$), but not TTTD of AEE ($P = 0.118$).

The ATTD of GE, ATTD of AEE, and TTTD of AEE was increased for a CO-based diet compared with CNTR and TAL-based diet ($P \leq 0.012$; Table 3.4). Barrows on the CNTR diet had decreased ATTD of AEE than a TAL-based diet, but the difference between the 2 diets was not evident for TTTD of AEE ($P < 0.050$).

Environment and Dietary Fat Effects on Belly, Carcass, and Loin Characteristics

No interactions between E × DF were evident for any belly, carcass, or loin characteristics ($P \geq 0.215$; Table 3.5). The HCW and back fat was greater for TN carcasses than both PFTN and HS carcasses ($P \leq 0.011$). Carcasses from PFTN pigs tended to yield less ($P =$

0.096) and have increased fat free lean ($P = 0.089$). Loin depth was unaffected by environmental treatment ($P = 0.261$). The 3% CO diets resulted in decreased loin depth ($P = 0.006$), but HCW, yield, back fat depth, and fat free lean, were unaffected by dietary fat ($P \geq 0.129$).

Loin characteristics were unaffected by E × DF ($P \geq 0.495$; Table 3.5). Bellies from TN barrows had increased weight, middle thickness, and a* values ($P \leq 0.029$), and tended to have increased edge thickness ($P = 0.055$) than PFTN and HS bellies. Bellies from PFTN and HS barrows had increased L* values than TN bellies ($P = 0.021$). Environment did not affect b* values or belly firmness ($P \geq 0.243$). Bellies from barrows fed a CO-based diet were heavier than bellies from those fed a TAL-based diet ($P = 0.018$). However, belly thickness, fat color, nor belly firmness was unaffected by dietary fat ($P \geq 0.215$).

Environment and Dietary Fat Effects on Fatty Acid Profile and Calculated Carcass Iodine Value

Oleic acid (**C18:1**) concentrations in jowl fat on d 7 collected from HS barrows tended to be less when fed either a CO-based diet or a TAL-based diet, but was greater in concentration when no additional fat was added in comparison to PFTN, resulting in a E × DF interaction ($P = 0.063$; Table 3.6). The sum of other minor saturated fatty acids increased in TN and HS pigs compared with PFTN ($P = 0.014$). Additionally, myristic acid tended to be greater in concentration in TN and HS jowl fat than PFTN ($P = 0.055$). The sum of other minor unsaturated fatty acids tended to increase in concentration in TN jowl fat ($P = 0.060$). Three percent TAL increased the concentration of eicosatrienoic acid ($P = 0.039$), while 3% CO tended to increase the concentration of linoleic acid (**C18:2**) ($P = 0.093$) in jowl fat collected on d 7.

Environment or dietary fat did not alter IV, unsaturated to saturated fatty acid ratio (**U:S**), or omega-3 to omega-6 fatty acid ratio (**n-3:n-6**; $P \geq 0.167$).

In jowl fat collected on d 21 and d 35, no E × DF interactions were evident for fatty acid concentrations, IV, U:S, or n-3:n-6, and none of these parameters were impacted by environmental treatment ($P \leq 0.102$; Table 3.7 and 3.8). On d 21, C18:1 decreased ($P = 0.022$; Table 3.7), but C18:2 increased ($P < 0.001$) in barrows fed CO-based diets. These changes on d 21 caused jowl IV to increase and n-3:n-6 to decrease ($P < 0.001$); the U:S ($P = 0.063$) tended to decrease in barrows fed CO. On d 35, the use of 3% dietary CO resulted in decreased C18:1 ($P < 0.001$; Table 3.8). Feeding a CO-based diet also increased linoleic, linolenic and eicosadienoic acid concentrations in jowl fat on d 35 ($P \leq 0.003$). These effects on d 35 caused jowl IV to increase and n3:n6 to decrease ($P < 0.001$).

Environment and Dietary Fat Effects on mRNA Abundance in Adipose Tissue

Interactions between E × DF were not evident for the mRNA abundance of *ACLY*, *ACSS2*, *ACACA*, *FASN*, *SCD*, *FADS2*, *EVOLV6*, *PRKAG1*, *PLIN1*, *ATGL*, *HSL*, and *INSR* in adipose tissue collected on d 7 ($P \geq 0.150$; Table 3.9). After 7 d of environmental treatment, the mRNA abundance of *ATGL* and *HSL* were less abundant in TN and HS barrows than in PFTN barrows ($P \leq 0.041$). The abundance of *SCD* mRNA was increased in HS barrows compared to TN barrows ($P = 0.047$). After 7 d of dietary treatment, mRNA abundance of *FASN* and *SCD* decreased in adipose tissue from barrows fed CO compared with barrows consuming the CNTR and TAL diets ($P \leq 0.011$; Table 3.10).

Discussion

Pigs dissipate heat poorly, are highly insulated, lack functional sweat glands, and are densely housed during late finishing causing a high risk of susceptibility to HS (White et al., 2008; Qu et al., 2015). Heat stress imposes substantial changes in the physiological status of pigs, such as acid-base homeostasis (Patience et al., 2005) and is noted for suppressing feed intake (Hao et al., 2014; Pearce et al., 2014) and therefore energy intake of the pig (Renaudeau et al., 2013). Heat stress has a greater impact on pigs with a high rate of lean gain, resulting in reduced carcass lean gain and protein accretion (Nienaber et al., 1997; Brown-Brandl et al., 2000). Due to HS shifting the ratio of protein accretion to lipid deposition ratio and the reduced protein accretion rate, the AA requirement for TN pigs is different than HS pigs (Nienaber et al., 1997; Kerr et al., 2003).

To alleviate HS suppressing feed intake, producers typically formulate diets on seasonal basis using ingredients with a low heat increment and greater energy density during the summer months (Stahly et al., 1981). Dietary fats and oils are ideal in meeting this ingredient description (Forbes and Swift, 1944; Kerr et al., 2015), and are therefore used more frequently and at higher dietary concentrations during the seasonally warm periods of the year. Unexpectedly, the data reported herein show that the pig's response to dietary fat is similar whether housed in a TN or a HS environment. Therefore, these data indicate that producers can anticipate that the inclusion of dietary fat in HS conditions will result in the same outcomes as including dietary fat in TN conditions.

However, it must be noted that while HS suppressed dietary energy intake by approximately 30% in comparison to contemporaries raised in TN conditions, the energy intake of HS barrows was still relatively high for this size of pig (Patience, 2012). This high energy

intake is probably due to this experiment being conducted using pigs with a high health status housed in individual pens, where other stressors outside of ambient room temperature were kept to a minimum (White et al., 2015).

Certainly, the response to dietary energy intake is not easy to predict (Collins et al., 2009; Beaulieu et al., 2009), and it has been recently suggested that pigs that consume less energy are more likely to respond to increases in dietary energy concentration (Patience, 2012). Therefore, the data reported herein should be complemented with data collected under differing feed intake conditions, including those representative of the industry, where daily ME intake for pigs of this size may be between 9.0 (Graham et al., 2014) and 9.7 Mcal ME/d (Kellner et al., 2016).

Heat stress barrows had decreased mRNA abundance of genes involved in the lipolytic cascade (adipose triglyceride lipase and hormone sensitive lipase), which was similarly found by Sanz Fernandez et al. (2015a). These lipases hydrolyze fatty acids from the stored triglycerides in adipose tissue to be utilized as energetic fuel for protein accretion and maintenance processes throughout the body (Zimmermann et al., 2004). This result provides mechanistic evidence as to why HS pigs have decreased muscle mass and increased adiposity, a phenotype which has been demonstrated in HS pigs for nearly half a century (Close and Mount, 1971; Bridges et al., 1998). However, we did not find any upstream alteration of the lipolysis pathway via quantifying mRNA abundance of the *AMPK* regulatory subunit which has be implicated in regulating lipolytic lipases (Gaidhu et al., 2012; Sanz Fernandez et al., 2015a). The retention of stored triglycerides in adipose tissue during HS when energy intake is decreased is the opposite of what occurs during TN conditions when energy intake is decreased; unexpectedly, under TN conditions, there is a classic catabolic response where stored lipids are mobilized and circulating non-esterified fatty acid concentrations and whole-body oxidation is increased (Vernon, 1992).

Reduced lipolysis in adipose tissue may be an attempt to reduce thermogenesis during mitochondrial fatty acid transport and beta-oxidation (Mujahid and Furuse, 2008). Another potential explanation, is insulin, an acute anabolic and anti-lipolytic hormone, which increased concentrations have been reported in a variety of species during HS (Baumgard and Rhoads, 2013).

Previous research has indicated that HS in pigs is not simply a suppression of lipolysis, it directly suppresses protein accretion and the rate of lean carcass gain (Neinaber et al., 1997), and results in a whole-body alteration of nutrient partitioning to a phenotype of increased adiposity due to increased insulin activity (Pearce et al., 2013; Sanz Fernandez et al., 2015a, b). An increase in whole-body insulin action is a conserved HS response across a multitude of species (Baumgard and Rhodes, 2013). Recent findings support this whole-body change in HS pigs. For example, Qu et al. (2015) found that HS increased the expression of genes involved in de novo lipogenesis and fatty acid uptake in adipose tissue, and Sanz Fernandez et al. (2015b) found HS increased whole-body insulin sensitivity. Furthermore, in utero HS alters the hierarchy of future nutrient partitioning resulting in a fatter phenotype at market (Johnson et al., 2015).

The direction of storing recently digested dietary lipids and retaining stored body lipids versus mobilizing and then utilizing lipids as an energy source for protein deposition and maintenance processes may explain why HS pigs are less caloric efficient. The energetic cost of a gram of deposited lipid is approximately 1.6 kcal of ME more than a gram of deposited protein (van Milgen and Noblet, 2003; Barea et al., 2010; Patience, 2012).

Despite HS altering lipid metabolism and increasing mRNA abundance of stearoyl CoA desaturase (delta-9-desaturase) in adipose tissue, HS had no significant effect on the carcass IV and fatty acid composition on d 7, 21, or at market (d 35). This suggests that any seasonal pork

fat quality issues are most likely due to decreased carcass weight and belly weight and thickness and not due to HS resulting in carcass fat with increased concentrations of unsaturated fatty acids. A recent finding by Seibert et al. (2015) demonstrated that adipose tissue of HS pigs contained a greater percentage of water than their TN contemporaries; which is consistent with pigs that are limit fed or leaner in phenotype having less lipid relative to water, indicative of small adipocyte size (Gnaedinger et al., 1963). Seibert et al. (2015) also reported that exposure to HS did not alter the fatty acid profile of adipose tissue. Similar to the data reported herein, White et al. (2008) found that when stocking density was adequate, HS increased stearoyl CoA desaturase mRNA abundance, but did not alter fatty acid synthase or carcass IV. However, when floor space was reduced from 0.93 m^2/pig to 0.66 m^2/pig in combination with HS, there was a further decrease in energy intake, and a significant increase in adipose tissue stearoyl CoA desaturase mRNA abundance, fatty acid synthase mRNA abundance, and carcass IV by approximately 4 g/100g (White et al., 2008). Under commercial stocking densities (eg, 0.70 m^2/pig) carcass IV values can be 2 to 10 g/100g greater than individually fed pigs under TN conditions (Kellner et al., 2016). Thus, HS pigs densely stocked in commercial production maybe at a greater risk of falling short of carcass IV standards than these data here in indicate. An interaction between stocking density and HS was also reported to reduce rate of gain (Kerr et al., 2005). In sum, these studies suggest that if HS pigs have adequate floor space and additional stressors are minimal, the pig can sustain a minimum level of energy intake such that no impact of carcass IV will be evident.

Pigs that are limit fed have been noted to have carcasses that are leaner and have greater carcass IVs (Madsen et al., 1992). The data herein agree with this phenotype as the PFTN carcasses tended to be leaner and had numerically higher carcass IVs than TN and HS carcasses.

Since the first demonstration by Ellis and Isbell (1923), it has become accepted that the fatty acid composition of carcass fat will be highly reflective of the dietary fatty acid composition (Apple et al., 2009; Kellner et al., 2015). The data reported herein reveal that the degree of unsaturation in dietary fat also modulate genes involved in de novo lipogenesis (Jump, 2002; Duran-Montge et al. 2009). Use of an unsaturated dietary fat (CO) versus a saturated fat (TAL) increased the mRNA abundance of fatty acid synthase. It has been demonstrated that dietary saturated fatty acids, in comparison with unsaturated fatty acids and in particular omega-6 fatty acids, suppress fatty acid synthase and de novo lipogenesis (Waterman et al.; 1975, Kouba et al., 1999; Duran-Montge et al, 2009). Dietary saturated fatty acids suppressing lipogenesis is not always a consistent response as Hsu et al. (2004) has shown; in their study, the mRNA abundance of fatty acid synthase was similar between diets with TAL or docosahexaenoic acid. Similarly, Allee et al. (1972) showed that CO and TAL suppressed lipogenesis to the same degree. De novo lipogenesis in the pig synthesizes saturated or monounsaturated fatty acids of either 16 or 18 carbons (Kloreag et al., 2007). Thus, feeding a saturated fat source would suppress the further production of similar saturated and monounsaturated fatty acids via lipogenesis and feeding an unsaturated dietary fat source would not have the same effect.

Heat stress has been reported to compromise the pig's intestinal integrity and morphology (Pearce et al., 2014); these negative effects are largely independent of reduced feed intake (Pearce et al., 2015). The data reported herein indicates the differences between HS and TN barrows in terms of the ATTD of GE and AEE were minimal after 17 d of HS exposure, and that there was no significant difference evident for TTTD of AEE. The use of CO resulted in greater ATTD of GE and AEE and TTTD of AEE. The increase in digestibility of a more unsaturated

dietary fat source versus a saturated fat source has been previously shown (Wiseman et al., 1990; Kerr et al., 2009; Kil et al., 2010). However, more work is needed to validate if unsaturated dietary fat sources have increased levels of DE and ME than saturated fat sources (Powels et al., 1995; NRC, 2012).

In conclusion, HS does not alter the pig's response to dietary fat. However, HS results in reduced growth, feed intake, caloric and feed efficiency, and a suppression of mRNA abundance of genes involved in the lipolytic cascade which results in fatter carcasses.

Literature cited

Acosta Camargo, J. A., R. D. Boyd, and J. F. Patience. 2015. Endogenous and exogenous fat digestion in growing pigs. J. Anim. Sci. 93(Suppl. 2):75.

Allee, G. L., D. R. Romsos, G. A. Leveille, and D. H. Baker. 1972. Lipogenesis and enzymatic activity in pig adipose tissue as influenced by source of dietary fat. J. Anim. Sci. 35:41–47. doi:10.2527/jas1972.35141x.

American Oil Chemists' Society (AOCS). 1998. Recommended practice Cd 1c-85. In: Official methods and recommended practices of the AOCS. 5th ed. AOCS, Campaign, IL.

AOAC. 2007. Official methods of analysis. 18th rev. ed. AOAC Int., Gaithersburg, MD.

Apple, J. K., C. V. Maxwell, D. L. Galloway, S. Hutchison, and C. R. Hamilton. 2009. Interactive effects of dietary fat source and slaughter weight in growing-finishing swine: II. Fatty acid composition of subcutaneous fat. J. Anim. Sci. 87:1423–1440. doi:10.2527/jas.2008-1454.

Barea, R., S. Dubios, H. Gilbert, P. Sellier, J. van Milgen, and J. Noblet. 2010. Energy utilization in pigs selected for high and low residual feed intake. J. Anim. Sci. 88:2062–2072. doi:10.2527/jas.2009-2395.

Baumgard, L. H., and R. P.Rhoads. 2013. Effects of heat stress on postabsorptive metabolism and energetics. Annu. Rev. Anim. Biosci. 1:311–337. doi:10.1146/annurev-animal-031412-103644.

Baumgard, L. H., R. P. Rhoads, M. L. Rhoads, N. K. Gabler, J. W. Ross, A. F. Keating, R. L. Boddicker, S. Lenka, and V. Sejian. 2012. Impact of climate change on livestock production. In: V. Sejian, S. M. K. Naqvi, T. Ezeji, J. Lakritz, and R. Lal, editors,

Environmental stress and amelioration in livestock production. Springer-Verlag, Berlin, Germany. p. 413–468. doi:10.1007/978- 3-642-29205-7_15.

Beaulieu, A. D., N. H. Williams, and J. F. Patience. 2009. Response to dietary digestible energy concentration in growing pigs fed cereal grain-based diets. J. Anim. Sci. 87:965–976. doi:10.2527/jas.2007-0834.

Bridges, T. C., L. W. Turner, and R. S. Gates. 1998. Economic evaluation of misting-cooling systems for growing/finishing swine through modeling. Appl. Eng. Agric. 14:425–430.

Brown-Brandl, T. M., J. A. Nienaber, L. W.Turner, and J. T.Yen. 2000. Manual and thermal induced feed intake restriction on finishing barrows. I: Effects on growth, carcass, composition, and feeding behavior. Trans. ASAE 43:993–997. doi:10.13031/2013.2996.

Christensen, K. D. 1962. Foderfedtets indflydelse pa smorrets og flaeskets kvalitet. (In Danish.) Thesis, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Close, W. H., and L. E. Mount. 1971. Energy retention in the pig at several environmental temperatures and levels of feeding. Proc. Nutr. Soc. 30:33A–34A.

Collins, C. J., A. C. Philpotts, and D. J. Henman. 2009. Improving growth performance of finisher pigs with high fat diets. Anim. Prod. Sci. 49:262–267. doi:10.1071/EA08243.

Comberg, G., E. Stephan, and H. Spath. 1972. The reaction of German Landrace boar progeny groups to different climatic pen conditions. Pt. 3. Zuchtungskunde 44:402–415.

Duran-Montgé, P., P. K. Theil, C. Lauridsen, and E. Esteve-Garcia. 2009. Dietary fat source affects metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. Animal 3:535–542. doi:10.1017/S1751731108003686.

Ellis, N. R., and H. S. Isbell. 1926. Soft pork studies. II. The influence of the character of the ration upon the composition of the body fat of hogs. J. Biol. Chem. 59:219–248.

Forbes, E. B., and R. W. Swift. 1944. Associative dynamic effects of protein, carbohydrate and fat. Science 99:476–478. doi:10.1126/science.99.2580.476.

Gaidhu, M. P., G. Bikopoulos, and R. B. Ceddia. 2012. Chronic AICAR-induced AMP-kinase activation regulates adipocyte lipolysis in a time-dependent and fat depot-specific manner in rats. Am. J. Physiol.: Cell Physiol. 303:C1192–C1197. doi:10.1152/ajpcell.00159.2012.

Graham, A. B., R. D. Goodband, M. D. Tokach, S. S. Dritz, J. M. DeRouchey, and S. Nitikanchana. 2014. The effects of mediumoil dried distillers grains with solubles on growth performance, carcass traits, and nutrient digestibility in growing-finishing pigs. J. Anim. Sci. 92:604–611. doi:10.2527/jas.2013-6798.

Gnaedinger, R. H., A. M. Pearson, E. P. Reineke, and V. M. Hix. 1963. Body composition of market weight pigs. J. Anim. Sci. 22:495– 500. doi:10.2527/jas1963.222495x.

Hao, Y., Y. Feng, P.Yang, J. Feng, H. Lin, and X. Gu. 2014. Nutritional and physiological responses of finishing pigs exposed to a permanent heat exposure during three weeks. Arch. Anim. Nutr. 68:296–308. doi:10.1080/1745039X.2014.931522.

Heitman, H., C. F. Kelly, and T. E. Bond. 1958. Ambient air temperature and weight gain in swine. J. Anim. Sci. 17:62–67. doi:10.2527/jas1958.17162x.

Hsu, J. M., P. H. Wang, B. H. Liu, and S. T. Ding. 2004. The effect of docosahexaenoic acid on the expression of porcine lipid metabolism-related genes. J. Anim. Sci. 82:683–689.

Johnson, J. S., M. V. Sanz Fernandez, J. F. Patience, J. W. Ross, N. K. Gabler, M. C. Lucy, T. J. Safranski, R. P. Roads, and L. H. Baumgard. 2015. Effects of in utero heat stress on postnatal body composition in pigs: II. Finishing phase. J. Anim. Sci. 93:82–92. doi:10.2527/jas.2014-8355.

Jump, D. B. 2002. Dietary polyunsaturated fatty acids and regulation of gene transcription. Curr. Opin. Lipidol. 13:155–164. doi:10.1097/00041433-200204000-00007.

Kellner, T. A., K. J. Prusa, and J. F. Patience. 2014. Impact of dietary fat source and concentration and daily fatty acid intake on the composition of carcass fat and iodine value sampled in three regions of the pork carcass. J. Anim. Sci. 92:5485–5495. doi:10.2527/jas.2014-7567.

Kellner, T. A., K. J. Prusa, and J. F. Patience. 2015. The impact of dietary fat withdrawal on carcass iodine value, belly characteristics, and changes in body fat over time. J. Anim. Sci. 93:247– 257. doi:10.2527/jas.2014-7650.

Kellner, T. A., S. Wisdom, G. G. Gourley, and J. F. Patience. 2016. Impact of increasing dietary energy concentration through graded levels of corn oil or choice white grease on carcass characteristics in pigs. J. Anim. Sci. 94(Suppl. 2):104. doi:10.2527/msasas2016-221.

Kerr, B. J., T. A. Kellner, and G. C. Shurson. 2015. Characteristics of lipids and their feeding value in swine diets. J. Anim. Sci. Biotechnol. 6:30. doi:10.1186/s40104-015-0028-x.

Kerr, B. J., T. E. Weber, W. A. Dozier III, and M. T. Kidd. 2009. Digestible and metabolizable energy content of crude glycerin originating from different sources in nursery pigs. J. Anim. Sci. 87:4042–4049. doi:10.2527/jas.2008-1676.

Kerr, B. J., J. T.Yen, J. A. Nienaber, and R. A. Easter. 2003. Influences of dietary protein level, amino acid supplementation and environmental temperature on performance, body composition, organ weights and total heat production of growing pigs. J. Anim. Sci. 81:1998–2007. doi:10.2527/2003.8181998x.

Kerr, C. A., L. R. Giles, M. R. Jones, and A. Reverter. 2005. Effects of grouping unfamiliar cohorts, high ambient temperature and stocking density on live performance of growing pigs. J. Anim. Sci. 83:908–915.

Kil, D. Y., T. E. Sauber, D. B. Jones, and H. H. Stein. 2010. Effect of the form of dietary fat and the concentration of dietary neutral detergent fiber on ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. J. Anim. Sci. 88:2959–2967. doi:10.2527/jas.2009-2216.

Kloareg, M., J. Noblet, and J. van Milgen. 2007. Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. Br. J. Nutr. 97:35–44. doi:10.1017/S0007114507205793.

Kouba, M., M. Bonneau, and J. Noblet. 1999. Relative development of subcutaneous, intermuscular, and kidney fat in growing pigs with different body compositions. J. Anim. Sci. 77:622–629.

Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. J. Assoc. Off. Anal. Chem. 56:535–537.

Lepage, G., and C. Roy. 1986. Direct transesterification of all classes of lipids in a one-step reaction. J. Lipid Res. 27:114–120.

Madsen, A., K. Jacobsen, and H. P. Mortensen. 1992. Influence of dietary fat on carcass fat quality in pigs. A review. Acta Agric. Scand., Sect. A 42:220–225.

Mujahid, A., and M. Furuse. 2008. Central administration of corticotropin-releasing factor induces thermogenesis by changes in mitochondrial bioenergetics in neonatal chicks. Neuroscience 155:845–851. doi:10.1016/j.neuroscience.2008.06.026.

National Pork Producers Council (NPPC). 2000. Pork composition and quality assessment procedure. NPPC, Des Moines, IA.

Nienaber, J. A., G. L. Hahn, R. A. Eigenberg, R. L. Korthals, J. T. Yen, and D. L. Harris. 1997. Genetic and heat stress interaction effects on finishing swine. In: Proc. 5th Int. Livest. Environ. Symp. Minneapolis, MN. p. 1017–1023.

Noblet, J. and J. M. Perez. 1993. Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. J. Anim. Sci. 71:3389–3398. NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Oresanya, T. F., A. D. Beaulieu, E. Beltranena, and J. F. Patience. 2007. The effect of dietary energy concentration and total lysine/ digestible energy ratio on the growth performance of weaned pigs. Can. J. Anim. Sci. 87:45–55. doi:10.4141/A05-064.

Patience, J. F. 2012. The influence of dietary energy on feed efficiency in grow-finish swine. In: J. F. Patience, editor, Feed efficiency in swine. Wageningen Academic Publishers, Wageningen, the Netherlands. p. 101–129. doi:10.3920/978-90-8686-756-1_5.

Patience, J. F., J. F. Umboh, R. K. Chaplin, and C. M. Nyachoti. 2005. Nutritional and physiological responses of growing pigs exposed to a diurnal pattern of heat stress. Livest. Prod. Sci. 96:205–214. doi:10.1016/j.livprodsci.2005.01.012.

Pearce, S. C., N. K. Gabler, J. W. Ross, J. Escobar, J. F. Patience, R. P. Rhoads, and L. H. Baumgard. 2013. The effects of heat stress and plane of nutrition on metabolism in growing pigs. J. Anim. Sci. 91:2108–2118. doi:10.2527/jas.2012-5738.

Pearce, S. C., S. M. Lonergan, E. Huff-Lonergan, L. H. Baumgard, and N. K. Gabler. 2015. Acute heat stress and reduced nutrient intake alter intestinal proteomic profile and gene expression in pigs. PLoS One 10:e0143099. doi:10.1371/journal.pone.0143099.

Pearce, S. C., M. V. Sanz Fernandez, J. H. Hollis, L. H. Baumgard, and N. K. Gabler. 2014. Short-term exposure to heat stress attenuates appetite and intestinal integrity in growing pigs. J. Anim. Sci. 92:5444–5454. doi:10.2527/jas.2014-8407.

Powles, J., J. Wiseman, D. J. A. Cole, and S. Jagger. 1995. Prediction of the apparent digestible energy value of fats given to pigs. Anim. Sci. 61:149–154. doi:10.1017/S1357729800013631.

Qu, H., S. S. Donkin, and K. M. Ajuwon. 2015. Heat stress enhances adipogenic differentiation of subcutaneous fat depot-derived porcine stromovascular cells. J. Anim. Sci. 93:3832–3842. doi:10.2527/jas.2015-9074.

Renaudeau, D., G. Frances, S. Dubois, H. Gilbert, and J. Noblet. 2013. Effect of thermal heat stress on energy utilization in two lines of pigs divergently selected for residual feed intake. J. Anim. Sci. 91:1162–1175. doi:10.2527/jas.2012-5689.

Renaudeau, D., H. Gilbert, and J. Noblet. 2012. Effect of climatic environment on feed efficiency in swine. In: J. F. Patience, editor, Feed efficiency in swine. Wageningen Academic Publishers, Wageningen, the Netherlands. p. 183–210. doi:10.3920/978-90-8686-756-1_9.

Sanz Fernandez, M. V., J. S. Johnson, M. Abuajamieh, S. K. Stoakes, J. T. Seibert, L. Cox, S. Kahl, T. H. Elsasser, J. W. Ross, S. C. Isom, R. P. Rhoads, and L. H. Baumgard. 2015a. Effects of heat stress on carbohydrate and lipid metabolism in growing pigs. Physiol. Rep. 3:e12315. doi:10.14814/phy2.12315.

Sanz Fernandez, M. V., S. K. Stoakes, M. Abuajamieh, J. T. Seibert, J. S. Johnson, E. A. Horst, R. P. Rhoads, and L. H. Baumgard. 2015b. Heat stress increases insulin sensitivity in pigs. Physiol. Rep. 3:e12478. doi:10.14814/phy2.12478.

Seibert, J. T., M.Abuajamieh, M. V. Sans-Fernandez, J. S.Johnson, S. M. Lei, S. K. Stoakes, J. F. Patience, J. W. Ross, R. P. Rhoads, S. M. Lonergan, L. H. Baumgard, and R. C.Johnson. 2015. Effects of heat stress on adipose tissue fatty acid composition and moisture content in pigs. J. Anim. Sci. 93(Suppl. 2):175. (Abstr.)

Semen, D. L., W. N. Barron, and M. Matzinger. 2013. Evaluating the ability to measure pork fat quality for the production of commercial bacon. Meat Sci. 94:252–256.

Spencer, J. D., A. M. Gaines, E. P. Berg, and G. L. Allee. 2005. Diet modifications to improve finishing pig performance and pork quality attributes during periods of heat stress. J. Anim. Sci. 83:243–254.

Stahly, G., G. L. Cromwell, and J. R. Overfield. 1981. Interactive effects of season of year and dietary fat supplementation, lysine source and lysine level on the performance of swine. J. Anim. Sci. 53:1269–1277. doi:10.2527/jas1981.5351269x.

St-Pierre, N., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. J. Dairy Sci. 86:E52– E77. doi:10.3168/jds.S0022-0302(03)74040-5.

Sullivan, Z. M., M. S. Honeyman, L. R. Gibson, and K. J. Prusa. 2007. Effects of triticale-based diets on finishing pig performance and pork quality in deep-bedded hoop barns. Meat Sci. 76:428–437. doi:10.1016/j.meatsci.2006.12.002.

van Milgen, J., and J. Noblet. 2003. Partitioning of energy intake to heat, protein and fat in growing pigs. J. Anim. Sci. 81(Suppl. 2):86–93.

Vernon, R. G. 1992. Effects of diet on lipolysis and its regulation. Proc. Nutr. Soc. 51:397–408. doi:10.1079/PNS19920053.

Waterman, R. A., D. R. Romsos, A. C. Tsai, E. R. Miller, and G. A. Leveille. 1975. Influence of dietary sunflower oil and tallow on growth, plasma-lipids and lipogenesis in rats, pigs and chicks. Proc. Soc. Exp. Biol. Med. 150:347–351. doi:10.3181/00379727-150-39033.

White, H. M., B. T. Richert, A. P. Schinckel, J. R. Burgess, S. S. Donkin, and M. A. Latour. 2008. Effects of temperature stress on growth performance and bacon quality in grow-finish pigs housed at two densities. J. Anim. Sci. 86:1789–1798. doi:10.2527/jas.2007-0801.

White, R. R., P. S. Miller, and M. D. Hanigan. 2015. Evaluating equations estimating change in swine feed intake during heat and cold stress. J. Anim. Sci. 93:5395–5410. doi:10.2527/jas.2015-9220.

Wiseman, J., D. J. A. Cole, and B. Hardy. 1990. The dietary energy values of soy-bean oil, tallow, and their blends for growing/finishing pigs. Anim. Prod. 50:513–518. doi:10.1017/S0003356100005006.

Zimmermann, R., J. G. Strauss, G. Haemmerle, G. Schoiswohl, R. Birner-Gruenberger, M. Riederer, A. Lass, G. Neuberger, F. Eisenhaber, A. Hermetter, and R. Zechner. 2004. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 306:1383–1386. doi:10.1126/science.1100747.

Table 3.1. Ingredient composition (as-fed basis) of the experimental diets formulated with no added fat (control), 3% corn oil, or 3% tallow

Ingredient, %	Control	3% Corn oil	3% Tallow
Corn	84.36	79.74	79.74
Soybean meal (46.5% CP)	12.71	14.35	14.35
Corn oil	-	3.00	-
Tallow	-	-	3.00
Limestone	0.90	0.90	0.90
Monocalcium phosphate	0.56	0.53	0.53
Salt	0.50	0.50	0.50
L-lysine HCL	0.15	0.15	0.15
DL-methionine	-	0.01	0.01
L-threonine	0.01	0.01	0.01
Vitamin premix[1]	0.20	0.20	0.20
Trace mineral premix[2]	0.15	0.15	0.15
Titanium dioxide	0.40	0.40	0.40
Santoquin[3]	0.06	0.06	0.06
Formulated composition			
NE, Mcal/kg	2.54	2.67	2.67
Standard ileal digestible AA, %			
Lysine	0.61	0.64	0.64
Methionine	0.20	0.21	0.21
Methionine + Cysteine	0.41	0.42	0.42
Threonine	0.39	0.41	0.41
Tryptophan	0.12	0.12	0.12
Calculated composition			
Heat increment[4], Mcal	1.16	1.34	1.18
Analyzed composition			
DM, %	88.65	89.01	88.39
GE, Mcal/kg	3.81	4.01	3.95
ME[5], Mcal/kg	3.70	3.90	3.85
Crude protein (N × 6.25), %	13.16	13.56	13.55
Crude fat, %	3.18	6.21	6.22
Dietary fat IV[6], g/100g	-	123.0	41.8
Diet IV[7], g/100g	117.9	120.8	84.6
Diet IVP[8]	37.5	75.0	52.6

[1]Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.023 mg vitamin B12 per kg of diet.

[2]Provided 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kg of diet.

[3]Santoquin Mixture 6 (Feed and forage Anti-oxidant; NOVUS International, Saint Charles, MO).

[4]Heat increment = ME - NE

[5]ME = DE × [1.003 – (0.0021 × CP)] (Noblet and Perez, 1993).

[6]Iodine value (IV) determined via titration (Barrow-Agee Laboratories, LLC, Memphis, TN).

[7]Iodine value calculated from fatty acid composition: IV = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate concentration (AOCS, 1998).

[8]Iodine value product (IVP) = (IV of the dietary lipids) × (% dietary lipid) × 0.10 (Christensen, 1962; Madsen et al., 1992).

Table 3.2. Effects of ad libitum feed intake in thermal neutral conditions (TN)[1], pair feeding in thermal neutral conditions (PFTN)[1,2], or heat stress (HS)[3], additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on daily respiration rate (RR), rectal temperature (RT), growth performance, and feed efficiency d 0 to 35

	Environment					Dietary fat					E × DF[4]
	Treatment					Treatment					
Item	TN	PFTN	HS	SEM	*P*-value	CNTR	CO	TAL	SEM	*P*-value	*P*-value
Initial BW, kg[5]	101.5	99.9	100.5	0.9	0.406	100.6	101.2	100.0	0.9	0.644	0.903
Final BW, kg[6]	137.0	127.2	125.0	1.3	<0.001	129.5	131.1	128.6	1.3	0.366	0.867
RR, breaths/min	36.3[b]	34.2[b]	78.3[a]	1.6	<0.001	50.2	49.0	49.6	1.7	0.692	0.904
RT, °C	38.1[b]	38.2[b]	39.0[a]	0.1	<0.001	38.4	38.4	38.5	0.1	0.653	0.192
ADG, kg	1.03[a]	0.77[b]	0.72[b]	0.03	<0.001	0.83	0.87	0.83	0.03	0.492	0.413
ADFI, kg	3.46[a]	2.49[b]	2.49[b]	0.10	<0.001	2.89	2.82	2.72	0.10	0.124	0.978
G:F	0.301[ab]	0.319[a]	0.290[b]	0.013	0.006	0.292	0.314	0.303	0.013	0.073	0.500

[a-c]Within a row, least squares means lacking a common superscript letter differ due to effect of environment, $P < 0.05$.

[1]Constant thermal neutral environment of ~24.0°C.

[2]Limit-fed based on HS feed intake on the previous day

[3]Diunral heat stress environment of ~33.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h from d 0 to d 7, ~33.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 7 to d 14, ~34.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 14 to d 21, ~34.5°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 21 to d 28, and ~35.0°C between 0800 h to 2000 h and ~28.0°C 2000 h to 0800 h for d 28 to d 35.

[4]Probability value for environment × dietary fat interaction (E × DF).

[5]d 0.

[6]d 35.

Table 3.3. Effects of ad libitum feed intake in thermal neutral conditions (TN)[1], pair feeding in thermal neutral conditions (PFTN)[1,2], or heat stress (HS)[3], additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on energy intake and caloric efficiency

	Environment					Dietary fat					E × DF[4]
	Treatment					Treatment					
Item	TN	PFTN	HS	SEM	*P*-value	CNTR	CO	TAL	SEM	*P*-value	*P*-value
ME intake, Mcal/d	13.1[a]	9.6[b]	9.5[b]	0.4	<0.001	10.7	11.0	10.4	0.4	0.090	0.990
ME intake:BW gain	12.8[ab]	12.2[b]	13.4[a]	0.7	0.013	12.8	12.6	13.0	0.7	0.654	0.477
ME intake:carcass gain	17.2[ab]	16.6[b]	18.1[a]	1.0	0.021	17.4	17.1	17.5	1.0	0.786	0.509

[a-c]Within a row, least squares means lacking a common superscript letter differ due to effect of environment, $P < 0.05$.

[1]Refer to Footnote 1 in Table 3.2.

[2]Refer to Footnote 2 in Table 3.2.

[3]Refer to Footnote 3 in Table 3.2.

[4]Probability value for environment × dietary fat interaction (E × DF).

Table 3.4. Effects of ad libitum feed intake in thermal neutral conditions (TN)[1], pair feeding in thermal neutral conditions (PFTN)[1,2], or heat stress (HS)[3], additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on apparent total tract digestibility (ATTD)[4] and true total tract digestibility (TTTD)[5] of DM, GE, and acid hydrolyzed ether extract (AEE)

	Environment					Dietary fat					E × DF[6]
	Treatment					Treatment					
Item	TN	PFTN	HS	SEM	*P*-value	CNTR	CO	TAL	SEM	*P*-value	*P*-value
ATTD, %											
DM	88.0	88.7	88.4	0.2	0.223	88.4	88.5	88.2	0.2	0.524	0.253
GE	88.2^{b}	89.1^{a}	88.8^{a}	0.2	0.008	88.4^{y}	89.1^{x}	88.6^{y}	0.2	0.011	0.525
AEE	60.2	61.4	59.0	0.8	0.055	41.5^{z}	71.2^{x}	67.8^{y}	0.8	<0.001	0.886
TTTD, %											
AEE	97.9	98.5	96.7	0.7	0.118	97.3^{y}	99.3^{x}	96.3^{y}	0.7	0.012	0.932

[a-c]Within a row, least squares means lacking a common superscript letter differ due to effect of environment, $P < 0.05$.

[x-z]Within a row, least squares means lacking a common superscript letter differ due to effect of dietary fat, $P < 0.05$.

[1]Refer to Footnote 1 in Table 3.2.

[2]Refer to Footnote 2 in Table 3.2.

[3]Refer to Footnote 3 in Table 3.2.

[4]Apparent total tract digestibility (ATTD; %) of either AEE, DM, or GE was calculated as 100 – {100 × [concentration (g) of TiO_2 in diet × concentration of (g) of AEE, DM, or GE in feces]/[concentration (g) of TiO_2 in feces × concentration of AEE, DM, or GE in diet]}; (Oresanya et al. 2007).

[5]Calculated via correcting ATTD of AEE for endogenous fat losses at 20 g of AEE/kg of DM intake.

[6]Probability value for environment × dietary fat interaction (E × DF).

Table 3.5. Effects of ad libitum feed intake in thermal neutral conditions (TN)[1], pair feeding in thermal neutral conditions (PFTN)[1,2], or heat stress (HS)[3], additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on carcass characteristics

	Environment					Dietary fat					E × DF[4]
	Treatment					Treatment					
Item	TN	PFTN	HS	SEM	*P*-value	CNTR	CO	TAL	SEM	*P*-value	*P*-value
HCW, kg	101.5[a]	93.1[b]	92.1[b]	1.2	<0.001	95.2	96.5	95.1	1.2	0.554	0.827
Yield, %	74.1	73.2	73.7	0.4	0.096	73.5	73.6	74.0	0.4	0.407	0.600
Loin depth, cm	6.23	5.95	5.97	0.23	0.261	6.11[x]	5.73[y]	6.30[x]	0.24	0.006	0.387
Back fat, cm	2.29[a]	1.99[b]	2.10[b]	0.21	0.011	2.19	2.14	2.06	0.21	0.353	0.854
Fat free lean, %	52.4	53.9	53.2	1.5	0.089	52.9	52.7	52.9	1.6	0.129	0.774
Loin characteristics											
Ultimate pH	5.6	5.6	5.6	0.1	0.873	5.6	5.6	5.7	0.1	0.199	0.640
LCS[5]	3.2	3.0	3.1	0.1	0.561	3.0	3.1	3.1	0.1	0.806	0.693
LMS[6]	1.8	1.8	1.7	0.1	0.495	1.7	1.7	1.8	0.1	0.829	0.515
Belly characteristics											
Belly weight, kg	8.6[a]	7.5[b]	7.8[b]	0.2	<0.001	8.0[xy]	8.3[x]	7.7[y]	0.2	0.018	0.372
Belly ET[7], cm	3.11	2.81	2.76	0.25	0.055	2.94	2.88	2.86	0.25	0.856	0.313
Belly MT[8], cm	2.47[a]	2.23[b]	2.20[b]	0.08	0.029	2.28	2.36	2.25	0.08	0.568	0.919
L*	71.8[b]	73.2[a]	73.4[a]	0.6	0.021	73.4	72.6	72.4	0.6	0.177	0.309
a*	11.6[a]	9.9[b]	10.4[b]	0.4	0.003	10.3	10.7	10.9	0.4	0.452	0.318
b*	7.7	7.3	7.4	0.2	0.303	7.3	7.6	7.5	0.2	0.210	0.215
Durometer	44.4	41.9	42.7	2.5	0.682	44.7	42.6	41.8	2.4	0.547	0.687
Belly firmness[9]	2.2	2.4	2.4	0.1	0.243	2.3	2.5	2.2	0.1	0.220	0.720

[a-c]Within a row, least squares means lacking a common superscript letter differ due to effect of environment, $P < 0.05$.

[x-z]Within a row, least squares means lacking a common superscript letter differ due to effect of dietary fat, $P < 0.05$.

[1]Refer to Footnote 1 in Table 3.2.

[2]Refer to Footnote 2 in Table 3.2.

[3]Refer to Footnote 3 in Table 3.2.

[4]Probability value for environment × dietary fat interaction (E × DF).

[5]LCS = loin color score; evaluated postmortem according to the Japanese color bar 1 to 6 scale, 1 = extremely light, 6 = extremely dark (Sullivan et al., 2007).

[6]LMS = loin marbling score; evaluated postmortem according to National Pork Board Standards (NPPC, 2000). The marbling standards correspond to percentage of intramuscular lipid.

[7]ET = edge thickness; measured in the middle scribe side of the belly.

[8]MT = middle thickness; measured in the middle of the belly.

[9]Measured by a subjective flop test with a score of 1, 2, or 3 with 1 being the firmest.

Table 3.6. Effects of ad libitum feed intake in thermal neutral conditions (TN)[1], pair feeding in thermal neutral conditions (PFTN)[1,2], or heat stress (HS)[3], additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on fatty acid profile and calculated iodine value (IV)[4] of jowl fat on d 7

	Environment					Dietary fat					E × DF[5]
	Treatment					Treatment					
Item	TN	PFTN	HS	SEM	*P*-value	CNTR	CO	TAL	SEM	*P*-value	*P*-value
Fatty acid[6], %											
C12:0, %	0.04	0.04	0.04	0.01	0.655	0.04	0.04	0.04	0.01	0.925	0.112
C13:0, %	0.04	0.04	0.04	0.01	0.623	0.04	0.04	0.04	0.01	0.936	0.372
C14:0, %	1.10	1.05	1.12	0.02	0.055	1.11	1.06	1.10	0.02	0.210	0.557
C15:0, %	0.04	0.04	0.04	0.01	0.516	0.03	0.04	0.04	0.01	0.592	0.398
C16:0, %	22.37	22.03	22.36	0.20	0.440	22.41	22.25	22.09	0.20	0.525	0.566
C16:1, %	2.44	2.22	2.32	0.13	0.270	2.46	2.29	2.23	0.13	0.169	0.848
C17:0, %	0.54	0.55	0.53	0.07	0.845	0.54	0.52	0.56	0.07	0.477	0.786
C17:1, %	0.36	0.36	0.37	0.04	0.882	0.37	0.35	0.38	0.04	0.323	0.372
C18:0, %	10.83	11.23	11.20	0.34	0.461	10.98	11.13	11.15	0.34	0.861	0.475
C18:1, %	44.36	44.61	43.66	0.35	0.101	44.50	43.63	44.50	0.35	0.140	0.063
C18:2, %	14.80	14.86	15.23	0.36	0.527	14.51	15.55	14.84	0.36	0.093	0.752
C18:3, %	0.63	0.64	0.67	0.03	0.117	0.64	0.66	0.65	0.03	0.556	0.957
C20:0, %	0.12	0.09	0.14	0.03	0.250	0.08	0.13	0.14	0.03	0.124	0.291
C20:1, %	0.93	0.92	0.90	0.06	0.468	0.93	0.89	0.93	0.06	0.305	0.495
C20:2, %	0.78	0.79	0.76	0.03	0.659	0.77	0.80	0.77	0.03	0.669	0.444
C20:3, %	0.11	0.09	0.09	0.01	0.369	0.07^{y}	0.10^{xy}	0.12^{x}	0.01	0.039	0.760
C22:1, %	0.30	0.30	0.30	0.02	0.958	0.29	0.30	0.29	0.02	0.814	0.450
Other SFA[7], %	0.15^{a}	0.11^{b}	0.14^{a}	0.02	0.014	0.13	0.13	0.13	0.02	0.939	0.186
Other UFA[8], %	0.07	0.05	0.04	0.01	0.060	0.05	0.05	0.06	0.01	0.795	0.194
U:S[9]	1.84	1.85	1.81	0.03	0.544	1.83	1.83	1.84	0.03	0.956	0.311
IV, g/100g	68.7	68.8	68.8	0.50	0.976	68.4	69.3	68.7	0.05	0.425	0.929
n-3:n-6[10]	0.049	0.048	0.049	0.003	0.563	0.048	0.048	0.050	0.003	0.167	0.757

[a-c]Within a row, least squares means lacking a common superscript letter differ due to effect of environment, $P < 0.05$.

[x-z]Within a row, least squares means lacking a common superscript letter differ due to effect of dietary fat, $P < 0.05$.

[1]Refer to Footnote 1 in Table 3.2.

[2]Refer to Footnote 2 in Table 3.2.

[3]Refer to Footnote 3 in Table 3.2.

[4]Iodine value was calculated by: [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate percentage concentration (AOCS, 1998).

[5]Probability value for environment × dietary fat interaction (E × DF).

[6]Lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), margaric acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), gadoleic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), docosenoic acid (C22:1).

[7]Saturated fatty acids.

[8]Unsaturated fatty acids.

[9]Unsaturated to saturated fatty acid ratio.

[10]Omega-3 fatty acid to Omega-6 fatty acid ratio.

Table 3.7. Effects of ad libitum feed intake in thermal neutral conditions (TN)[1], pair feeding in thermal neutral conditions (PFTN)[1,2], or heat stress (HS)[3], additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on fatty acid profile and calculated iodine value (IV)[4] of jowl fat on d 21

	Environment					Dietary fat					E × DF[5]
	Treatment					Treatment					
Item	TN	PFTN	HS	SEM	*P*-value	CNTR	CO	TAL	SEM	*P*-value	*P*-value
Fatty acid[6], %											
C12:0, %	0.05	0.04	0.04	0.01	0.102	0.05	0.04	0.04	0.01	0.479	0.829
C13:0, %	0.04	0.03	0.05	0.01	0.158	0.04	0.04	0.04	0.01	0.917	0.986
C14:0, %	1.13	1.07	1.12	0.03	0.109	1.12	1.10	1.10	0.03	0.785	0.454
C15:0, %	0.04	0.03	0.03	0.01	0.867	0.03	0.04	0.03	0.01	0.886	0.949
C16:0, %	22.22	21.80	21.90	0.20	0.370	22.18	21.73	22.00	0.20	0.294	0.768
C16:1, %	2.57	2.43	2.46	0.11	0.574	2.47	2.49	2.51	0.11	0.951	0.382
C17:0, %	0.49	0.47	0.51	0.05	0.540	0.48	0.48	0.51	0.05	0.496	0.264
C17:1, %	0.34	0.35	0.36	0.04	0.525	0.36	0.33	0.36	0.04	0.311	0.778
C18:0, %	10.44	10.49	10.42	0.36	0.970	10.64	10.12	10.58	0.36	0.162	0.662
C18:1, %	45.91	46.06	45.36	0.49	0.349	45.73^{xy}	45.01^{y}	46.60^{x}	0.49	0.022	0.251
C18:2, %	13.78	14.24	14.65	0.36	0.197	13.89^{y}	15.57^{x}	13.20^{y}	0.36	<0.001	0.473
C18:3, %	0.58	0.61	0.63	0.03	0.125	0.61	0.63	0.58	0.03	0.124	0.818
C20:0, %	0.11	0.09	0.12	0.03	0.659	0.10	0.09	0.14	0.03	0.420	0.810
C20:1, %	0.99	0.98	0.96	0.06	0.697	0.98	0.95	1.01	0.06	0.340	0.194
C20:2, %	0.72	0.77	0.75	0.04	0.696	0.73	0.77	0.74	0.04	0.717	0.159
C20:3, %	0.10	0.08	0.09	0.02	0.618	0.10	0.08	0.09	0.02	0.449	0.149
C22:1, %	0.26	0.27	0.27	0.02	0.848	0.26	0.27	0.27	0.02	0.857	0.310
Other SFA[7], %	0.12	0.11	0.13	0.01	0.238	0.13	0.12	0.12	0.01	0.537	0.508
Other UFA[8], %	0.07	0.05	0.05	0.01	0.134	0.05	0.05	0.06	0.01	0.804	0.169
U:S[9]	1.90	1.94	1.92	0.04	0.659	1.88	1.97	1.90	0.04	0.063	0.812
IV, g/100g	68.3	69.2	69.3	0.7	0.259	68.3^{y}	70.6^{x}	67.8^{y}	0.7	<0.001	0.960
n-3:n-6[10]	0.048	0.048	0.048	0.004	0.860	0.050^{x}	0.045^{y}	0.049^{x}	0.004	<0.001	0.146

[a-c]Within a row, least squares means lacking a common superscript letter differ due to effect of environment, $P < 0.05$.

[x-z]Within a row, least squares means lacking a common superscript letter differ due to effect of dietary fat, $P < 0.05$.

[1]Refer to Footnote 1 in Table 3.2.

[2]Refer to Footnote 2 in Table 3.2.

[3]Refer to Footnote 3 in Table 3.2.

[4]Iodine value was calculated by: [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate percentage concentration (AOCS, 1998).

[5]Probability value for environment × dietary fat interaction (E × DF).

[6]Lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), margaric acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), gadoleic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), docosenoic acid (C22:1).

[7]Saturated fatty acids.

[8]Unsaturated fatty acids.

[9]Unsaturated to saturated fatty acid ratio.

[10]Omega-3 fatty acid to Omega-6 fatty acid ratio.

Table 3.8. Effects of ad libitum feed intake in thermal neutral conditions (TN)[1], pair feeding in thermal neutral conditions (PFTN)[1,2], or heat stress (HS)[3], additional inclusion of no dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on fatty acid profile and calculated iodine value (IV)[4] of jowl fat on d 35

	Environment					Dietary fat					E × DF[5]
	Treatment					Treatment					
Item	TN	PFTN	HS	SEM	*P*-value	CNTR	CO	TAL	SEM	*P*-value	*P*-value
Fatty acid[6], %											
C12:0, %	0.04	0.04	0.04	0.01	0.315	0.04	0.04	0.04	0.01	0.913	0.710
C14:0, %	1.11	1.04	1.08	0.03	0.257	1.08	1.07	1.08	0.03	0.902	0.955
C15:0, %	0.04	0.04	0.03	0.01	0.294	0.03	0.04	0.04	0.01	0.054	0.168
C16:0, %	21.88	21.36	21.72	0.19	0.211	21.72	21.47	21.78	0.19	0.508	0.580
C16:1, %	2.39	2.24	2.36	0.08	0.338	2.41	2.26	2.31	0.08	0.327	0.477
C17:0, %	0.38	0.41	0.40	0.04	0.427	0.38	0.39	0.42	0.04	0.089	0.129
C17:1, %	0.36	0.38	0.36	0.03	0.492	0.36	0.36	0.38	0.03	0.302	0.162
C18:0, %	10.51	10.43	10.70	0.41	0.529	10.53	10.29	10.82	0.41	0.162	0.138
C18:1, %	45.88	46.59	45.99	0.45	0.497	47.14^{x}	44.65^{y}	46.67^{x}	0.46	<0.001	0.178
C18:2, %	14.40	14.41	14.30	0.37	0.961	13.73^{y}	16.30^{x}	13.44^{y}	0.37	<0.001	0.116
C18:3, %	0.62	0.64	0.64	0.02	0.707	0.60^{y}	0.68^{x}	0.61^{y}	0.03	0.003	0.533
C20:0, %	0.15	0.15	0.14	0.01	0.600	0.14	0.15	0.15	0.01	0.705	0.167
C20:1, %	0.94	0.96	0.94	0.03	0.767	0.97	0.92	0.95	0.03	0.351	0.245
C20:2, %	0.76	0.78	0.76	0.02	0.658	0.73^{y}	0.84^{x}	0.73^{y}	0.02	<0.001	0.494
C20:3, %	0.11	0.11	0.11	0.01	0.872	0.10	0.11	0.11	0.01	0.127	0.882
C22:1, %	0.27	0.27	0.28	0.01	0.649	0.26	0.29	0.27	0.01	0.082	0.304
Other SFA[7], %	0.11	0.11	0.11	0.01	0.839	0.11	0.11	0.11	0.01	0.965	0.269
Other UFA[8], %	0.03	0.05	0.03	0.01	0.260	0.03	0.03	0.05	0.01	0.068	0.645
U:S[9]	1.93	1.98	1.93	0.05	0.370	1.95	1.99	1.91	0.05	0.164	0.185
IV, g/100g	69.3	69.8	69.2	0.7	0.624	68.5^{y}	71.5^{x}	68.2^{y}	0.7	<0.001	0.197
n-3:n-6[10]	0.050	0.051	0.051	0.003	0.216	0.051^{y}	0.047^{z}	0.053^{x}	0.003	<0.001	0.115

[a-c]Within a row, least squares means lacking a common superscript letter differ due to effect of environment, $P < 0.05$.

[x-z]Within a row, least squares means lacking a common superscript letter differ due to effect of dietary fat, $P < 0.05$.

[1]Refer to Footnote 1 in Table 3.2.

[2]Refer to Footnote 2 in Table 3.2.

[3]Refer to Footnote 3 in Table 3.2.

[4]Iodine value was calculated by: [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.785 + [C22:1] × 0.723; brackets indicate percentage concentration (AOCS, 1998).

[5]Probability value for environment × dietary fat interaction (E × DF).

[6]Lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), margaric acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), gadoleic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), docosenoic acid (C22:1).

[7]Saturated fatty acids.

[8]Unsaturated fatty acids.

[9]Unsaturated to saturated fatty acid ratio.

[10]Omega-3 fatty acid to Omega-6 fatty acid ratio.

Table 3.9. Effects of ad-libitum feed intake in thermal neutral conditions (TN)[1], pair-feeding in thermal neutral conditions (PFTN)[1,2], or heat stress (HS)[3] on mRNA abundance in adipose tissue on d 7[4]

			Environment, $\Delta\Delta C_t$[5]				Fdiff[6]			
Gene	Description	Primers, 5`-3`	TN	PFTN	HS	SEM	TN vs. PFTN	HS vs. TN	HS vs. PFTN	*P*-value[7]
ACLY	ATP citrate lyase	F:AGGAGGAGTTCTATGTCTGC[8] R:CAACAGGTGTTTCTTGATGGCC[9]	0.30	-0.64	0.21	0.63	-1.91	1.06	-1.80	0.537
ACSS2	Acyl-CoA synthetase short-chain family member 2	F:TGTGAACCTGAAGGAGCTGG R:ACAATGCAGCATCTCACTGG	0.23	-0.38	-0.45	0.72	-1.52	1.60	1.05	0.633
ACACA	Acetyl CoA carboxylase	F:ATGGATGAACCGTCTCCC R:TGTAAGGCCAAGCCATCC	-0.20	-0.56	0.27	1.25	-1.28	-1.39	-1.78	0.517
FASN	Fatty acid synthase	F:CACAACTCCAAAGACACG R:AGGAACTCGGACATAGCG	-0.42	-0.23	-1.15	0.81	1.14	1.66	1.89	0.249
SCD	Stearoyl CoA desaturase (delta-9-desaturase)	F:TACTATCTGCTGAGTGCTGTGG R:CTGGAATGCCATCGTGTTGG	0.48[a]	-0.29[ab]	-2.13[b]	1.19	-1.71	6.11	3.58	0.047
FADS2	Fatty acid desaturase 2 (delta-6-desaturase)	F:GCCTTCATCCTTGCTACC R:AGATGGCCGTAATCGTGC	0.89	-1.02	0.33	1.35	-3.76	1.47	-2.55	0.295
EVOLV6	Fatty acid elongase 6	F:CTGGTTTCTGCTCTGTATGC R:ACCTGAACACTGCAAGGC	0.63	-0.31	0.80	0.81	-1.91	-1.13	-2.16	0.542
PRKAG1	Protein kinase, AMP-activated, gamma 1 non-catalytic subunit	F:TTGGTGACTAATGGTGTCCG R:TGAAATCAGTGATGGTCAGC	0.36	0.02	0.30	1.84	-1.27	1.04	-1.21	0.889
PLIN1	Perilipin 1	F:GAGTGCTTCCAGAAGACC R:GATGCCCTTCTCGTAAGC	0.35	0.45	-0.85	1.60	1.07	2.30	2.46	0.418
ATGL (PNPLA2)	Adipose triglyceride lipase (Patatin-like phospholipase domain containing 2)	F:ATCATAACCCACTTCGCC R:ACACGGGAATGAAGGTGC	0.08[a]	-1.80[b]	1.15[a]	0.88	-3.68	-2.10	-7.73	<0.001
HSL	Hormone sensitive lipase	F:AACGCAATGAAACAGGCC R:TGTATGATCCGCTCAACTCG	-0.01[b]	-0.36[b]	1.54[a]	1.53	-1.27	-2.93	-3.73	0.041
INSR	Insulin receptor	F:CGACCATCTGTAAGTCGC R:GTCTTGGAAGTGGTAGTAGG	-0.39	0.40	-0.02	0.81	1.73	-1.29	1.33	0.823

[a-c]Within a row, least squares means lacking a common superscript differ, $P < 0.05$.

[1]Refer to Footnote 1 in Table 2.

[2]Refer to Footnote 2 in Table 2.

[3]Refer to Footnote 3 in Table 2.

[4]No interaction between environment and dietary fat was evident ($P \geq 0.15$).

[5]Delta delta C_t.
[6]Fold difference: positive/negative values indicate increase/decrease mRNA abundance.
[7]Probability value for main effect of environment.
[8]Forward sequence.
[9]Reverse sequence.

Table 3.10. Effects of dietary fat (CNTR), 3% tallow (TAL), or 3% corn oil (CO) on mRNA abundance in adipose tissue on d 7[1]

			Dietary fat, $\Delta\Delta C_t$[2]				Fdiff[3]			
Gene	Description	Primers, 5`-3`	CNTR	TAL	CO	SEM	CNTR vs. TAL	CO vs. CNTR	CO vs. TAL	*P*-value[4]
ACLY	ATP citrate lyase	F:AGGAGGAGTTCTATGTCTGC[5] R:CAACAGGTGTTTCTT GATGGCC[6]	-0.04	0.78	-0.85	0.63	1.76	1.75	3.10	0.201
ACSS2	Acyl-CoA synthetase short-chain family member 2	F:TGTGAACCTGAAGGAGCTGG R:ACAATGCAGCATCTCACTGG	-0.81	0.52	-0.33	0.72	2.51	-1.39	1.80	0.215
ACACA	Acetyl CoA carboxylase	F:ATGGATGAACCGTCTCCC R:TGTAAGGCCAAGCCATCC	0.15	0.02	-0.66	1.25	-1.09	1.75	1.60	0.566
FASN	Fatty acid synthase	F:CACAACTCCAAAGACACG R:AGGAACTCGGACATAGCG	-0.36[a]	0.20[a]	-1.64[b]	0.81	1.47	2.43	3.58	0.011
SCD	Stearoyl CoA desaturase (delta-9-desaturase)	F:TACTATCTGCTGAGTGCTGTGG R:CTGGAATGCCATCGTGTTGG	0.11[a]	0.90[a]	-2.94[b]	1.18	1.72	8.28	14.32	0.002
FADS2	Fatty acid desaturase 2 (delta-6-desaturase)	F:GCCTTCATCCTTGCTACC R:AGATGGCCGTAATCGTGC	0.83	-0.49	-0.14	1.34	-2.50	1.96	-1.27	0.474
EVOLV6	Fatty acid elongase 6	F:CTGGTTTCTGCTCTGTATGC R:ACCTGAACACTGCAAGGC	1.16	0.45	-0.48	0.82	-1.63	3.11	1.91	0.309
PRKAG1	Protein kinase, AMP-activated, gamma 1 non-catalytic subunit	F:TTGGTGACTAATGGTGTCCG R:TGAAATCAGTGATGGTCAGC	0.69	0.21	-0.22	1.84	-1.39	1.88	1.35	0.444
PLIN1	Perilipin 1	F:GAGTGCTTCCAGAAGACC R:GATGCCCTTCTCGTAAGC	0.51	0.90	-1.46	1.60	1.31	3.92	5.13	0.101
ATGL (PNPLA2)	Adipose triglyceride lipase (Patatin-like phospholipase domain containing 2)	F:ATCATAACCCACTTCGCC R:ACACGGGAATGAAGGTGC	0.04	0.31	-0.92	0.88	1.21	1.95	2.35	0.258
HSL	Hormone sensitive lipase	F:AACGCAATGAAACAGGCC R:TGTATGATCCGCTCAACTCG	0.13	0.36	0.68	1.53	1.17	-1.46	-1.25	0.807
INSR	Insulin receptor	F:CGACCATCTGTAAGTCGC R:GTCTTGGAAGTGGTAGTAGG	0.91	-0.04	-0.88	0.81	-1.93	3.46	1.79	0.313

[a-c]Within a row, least squares means lacking a common superscript differ, $P < 0.05$.
[1]No interaction between environment and dietary fat was evident ($P \geq 0.15$).
[2]Delta delta C_t.
[3]Fold difference: positive/negative values indicate increase/decrease mRNA abundance.
[4]Probability value for main effect of dietary fat.

[5]Forward sequence.
[6]Reverse sequence.

Figure 3.1. Ambient room temperature (°C) by d during the 35 d experiment. Temperature was controlled to achieve a constant 24°C in the thermoneutral room which housed thermoneutral (TN) and pair-fed thermoneutral (PFTN) barrows. The heat stress room which housed the heat stress (HS) barrows was controlled to heat in a diurnal pattern at 28°C from 2000 h to 800 h and at 33°C d 0 to 7, 33.5°C d 7 to 14, 34°C d 14 to 21, 34.5°C d 21 to 28, 35°C d 28 to 35 from 800 h to 2000 h.

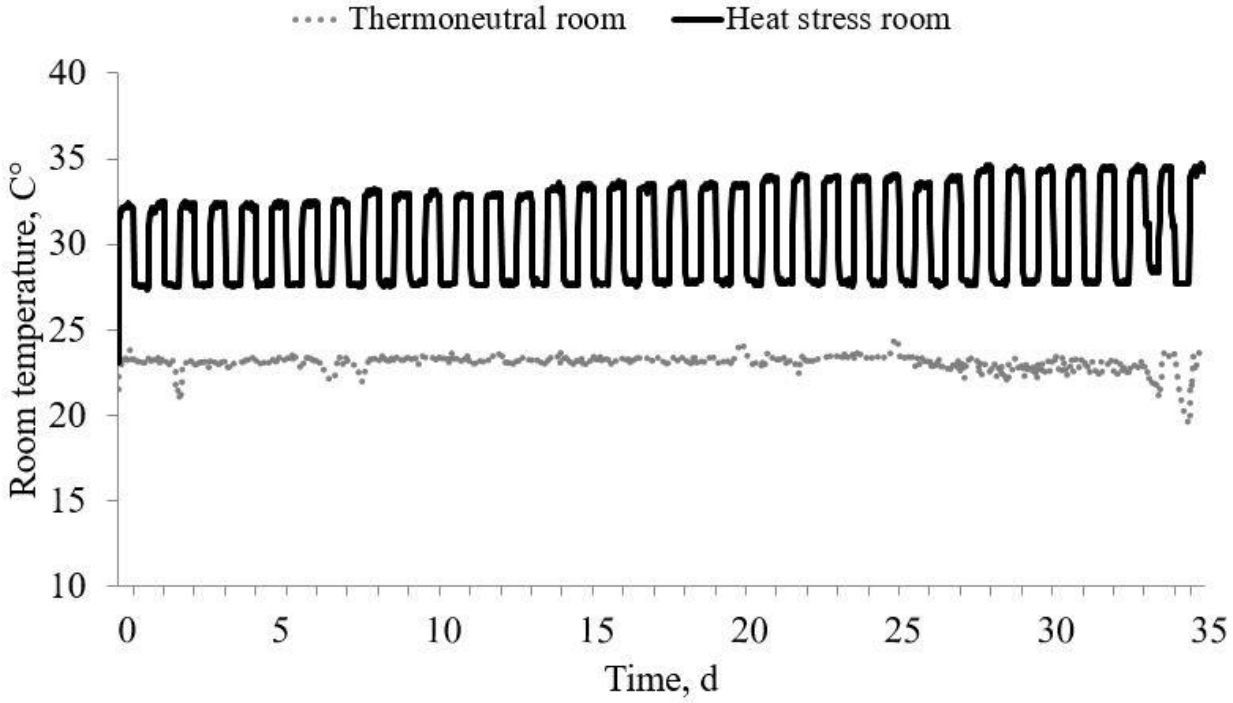
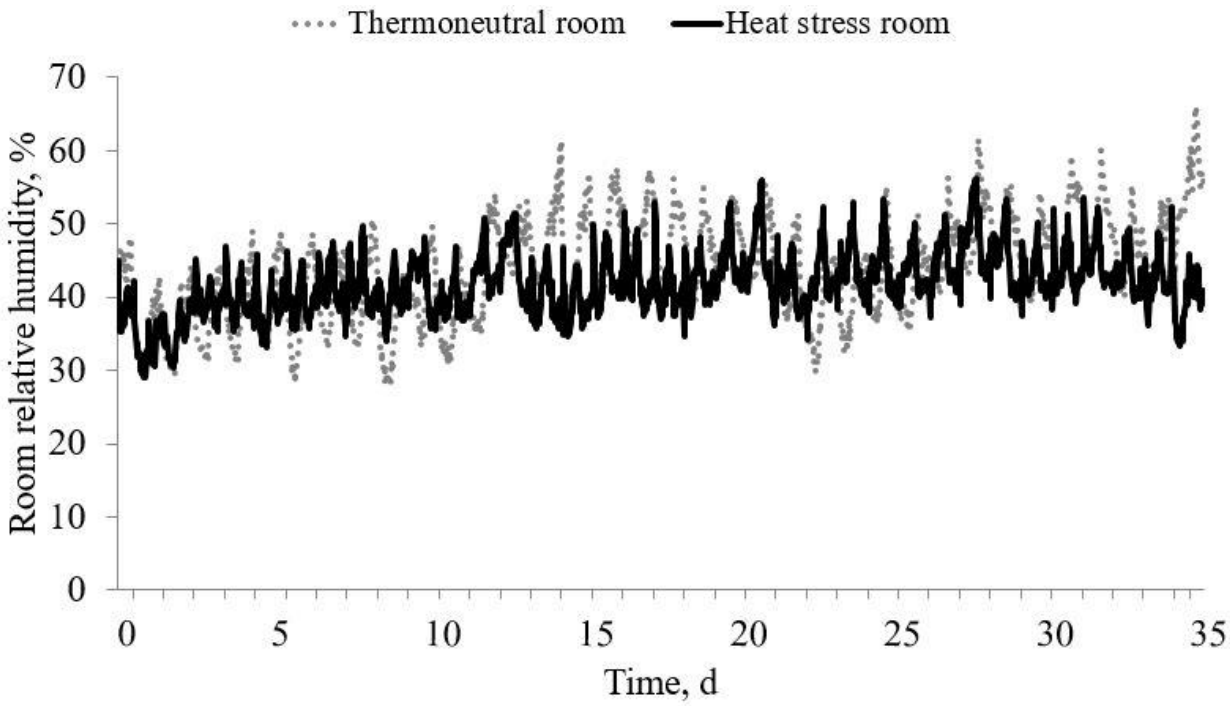


Figure 3.2. Relative humidity (%) of the room by d during the 35 d experiment. Humidity was not governed during the 35 d experiment. Thermoneutral room housed thermoneutral (TN) and pair-fed thermoneutral (PFTN) barrows, and the heat stress room housed heat stress (HS) barrows.



CHAPTER IV

THE COMPOSTION OF DIETARY FAT ALTERS THE TRANSCRIPTIONAL PROFILE OF PATHWAYS ASSOCIATED WITH LIPID METABOLISM IN THE LIVER AND ADIPOSE TISSUE IN THE PIG

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T. A. Kellner, N. K. Gabler, and J. F. Patience*

*Department of Animal Science, Iowa State University, Ames, 50011, USA

Abstract

The objective was to investigate the of effect chemical composition of dietary fat on transcription of genes involved in lipid metabolism in adipose tissue and liver via transcriptional profiling in growing pigs. A total of 48 Genetiporc 6.0 × Genetiporc F25 (PIC, Inc., Hendersonville, TN) barrows (initial BW of 44.1 ± 1.2 kg) were randomly allotted to 1 of 6 dietary treatments. Each experimental diet included 95% of a corn-soybean meal basal diet and 5% of either: corn starch (CNTR), animal-vegetable blend (AV), coconut oil (COCO), corn oil (COIL), fish oil (FO), or tallow (TAL). Pigs were sacrificed on d 10 (final BW of 51.2 ± 1.7 kg) to collect tissues. Expression normalization across samples was performed by calculating a delta Ct (ΔCt; cycle threshold) value using *RPL32*. Delta delta Ct values (ΔΔCt) were expressed relative to the CNTR treatment. In adipose tissue adding dietary fat regardless of source decreased the mRNA abundance of *FASN* compared to CNTR fed pigs ($P = 0.014$). Of the dietary fat sources tested, pigs fed a COIL based diet tended to have greater adipose tissue

expression of *FASN* ($P = 0.071$). Abundance of *PRKAG-1* mRNA was greater in adipose tissue of barrows a fed COIL based diet than barrows fed CNTR or FO diets ($P = 0.047$). In liver adding dietary fat regardless of source increased the mRNA abundance of *ACACA*, *ATGL*, *INSR*, *PPAR-α*, *PRKAG-1*, and *SCD* ($P \leq 0.020$) and tended to have greater abundance of *HSL* ($P = 0.071$) and *SREBP-1* ($P = 0.086$) compared to CNTR fed barrows. Pigs fed a TAL based diet had greater *HSL* hepatic transcription than pigs fed CNTR, COCO, or FO diets ($P = 0.013$). Hepatic transcription of *FASN* tended to be the greater in pigs fed COCO than pigs fed other dietary fat sources ($P = 0.074$). Dietary fat omega-3 content tended to negatively correlate with mRNA abundance of *PRKAG-1* ($P = 0.065$) in adipose tissue and *ATGL* ($P = 0.063$) in liver. Dietary fat SFA content was negatively correlated with *PPAR-α* in liver ($P \leq 0.039$). Dietary fat MUFA content tended to be positively correlated with *ACACA*, *PPAR-α*, *PRKAG-1* mRNA abundance in liver ($P \leq 0.100$). To conclude, the intake of omega-3 fatty acids suppressed the mRNA abundance of genes involved in lipolysis in both adipose tissue and liver. Dietary SFAs appear to be greater inhibitors of lipogenesis in adipose tissue than omega-6 fatty acids. Intake of medium chain fatty acids alter hepatic lipid metabolism differently than intake of long chain fatty acids.

Introduction

Increased inclusion of dietary fat is known to suppress lipogenesis in adipose tissue of pigs (Bortz et al., 1963; Allee et al., 1971). How dietary fat sources that differ in their fatty acid composition alter the transcription of genes involved in lipid metabolism is less known (Jump, 2002; Duran-Montge et al., 2009). Sources of dietary fat are diverse in fatty acid chain length and degree of unsaturation (Powles et al, 1995; NRC, 2012). Quantifying the effect of dietary fat

composition on gene abundance associated with lipid partitioning can provide insight into changes in post-absorptive lipid metabolism. This, in turn, would lead to a more accurate prediction of the pig's response to inclusion of fat into its diet.

Most published studies on lipid metabolism employed human or rodent subjects (Bergen and Mersmann, 2005), and of the few in growing pigs, most have measured the expression of genes involved in hepatic lipogenesis (Duran-Montge et al., 2009). Thus, there are few data to describe the effects of dietary fat source on lipogenesis and lipolysis in adipose tissue (O'Hea and Leveille, 1969). The current understanding derived from these few porcine studies is that SFA inhibit adipose lipogenesis more than omega-6 fatty acids (Smith et al., 1996; Kellner et al., 2016a). However, suppression of lipogenesis due to the intake of SFA is not a consistent finding (Allee et al., 1971). It has also been reported that dietary MUFA are positively correlated with increased hepatic lipogenesis (Duran-Montge et al., 2009). Thus, the objective was to investigate the effect of chemical composition of dietary fat sources on transcriptional profiling of genes involved in lipid metabolism in adipose tissue and liver in growing pigs.

Materials and methods

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research, and were approved by the Iowa State University Institutional Animal Care and Use Committee (#2-16-8201-S).

Animals, Housing, and Experimental Design

A total of 48 Genetiporc 6.0 × F25 (PIC, Inc., Hendersonville, TN) barrows (in 2 sequential cohorts of 24 barrows each) with an average initial BW of 44.1 ± 1.2 kg were

randomly allotted to 1 of 6 dietary treatments in a 10 d experiment. Pigs were housed individually throughout the experiment, in a room in which each pen provided 1.83 m^2 of floor space, a nipple drinker, and a composite feeder and had slatted concrete flooring. The length of experiment was based on previous data from Kellner et al. (2015, 2016a) showing that the mRNA abundance of genes involved in lipid metabolism and the fatty acid composition of depot fat in pigs can be altered by dietary fat intake within 7 to 14 d.

Diets and Feeding

Each experimental diet (Table 4.1) consisted of a corn-soybean meal diet with either 5% cornstarch (Control [**CNTR**] or 1 of 5 dietary fat sources: animal-vegetable blend (**AV** with iodine value [**IV**] = 68.7 g/100 g; Darling Pro Ingredients, Wahoo, NE), coconut oil (**COCO** with IV = 1.0 g/100 g; Bulk Apothecary, Aurora, OH), corn oil (**COIL** with IV = 126.3 g/100 g; Feed Energy Co., Des Moines, IA), fish oil (**FO** with IV = 137.4 g/100 g; Double S Liquid Feed Services, Danville, IL), or tallow (**TAL** with IV = 44.0 g/100 g; Darling Pro Ingredients, Omaha, NE). Dietary fat sources were selected to provide a diverse range of fatty acid profiles and degree of unsaturation. More specifically, COCO was selected to provide intake of saturated medium chain fatty acids. The COIL source was selected to provide a high intake of omega-6 fatty acids, while FO provide a high intake of omega-3 fatty acids. A TAL source was selected to provide a high intake of saturated and mono-unsaturated long chain fatty acids and a low intake of PUFA. Finally, an AV source was selected to provide a combination of SFA, MUFA, and PUFA intake. The chemical composition and the fatty acid profiles of the dietary fats are presented in Tables 4.2 and 4.3, respectively.

Feed was provided at 3.2 times maintenance (NRC, 2012). The daily feed allowance was provided in 2 equal meals at 0800 h and 1600 h. If any feed remained in the feeders at 0800 h, it was measured and discarded before the next allotment of feed was added. Daily energy intake (kcal of NE/d) was determined using the following equation: $[(BW^{0.6}) \times 197] \times 3.2$ (NRC, 2012). Prior to the initiation of the study, pigs were fed a common diet. Water was provided ab libitum.

Data and Sample Collection

Pigs were individually weighed on d 0, 7 and 10. Pigs were sacrificed on d 10 (final BW = 51.2 ± 1.7 kg) to collect tissue samples. Adipose tissue was collected using a cork bore (12.7 mm; Flinn Scientific, Bativia, IL) from the 10^{th} rib back fat (ensured all back-fat layers of adipose tissue were represented as in the pig). Immediately following the collection, all skin and lean tissue if present was removed from the cored sample. Jejunum tissue was collected by removing the small intestine and collecting a 10 cm section 5.0 to 5.1 m from the pyloric sphincter; the tissue was immediately rinsed with buffered saline to remove all digesta. Liver was collected by taking a 1 × 1 cm cross section from the middle of the right lobe. Post-collection, all tissue samples were immediately placed in a 7.6 × 17.8 cm labelled sterile sample bag (Fisher Science, Hanover Park, IL), snap frozen in liquid nitrogen, and stored at -80°C for later analysis.

Diet Analysis

Dietary fat sources were analyzed in duplicate at a commercial laboratory (Barrow-Agee Laboratories, Memphis, TN) for fatty acid content (method Ce 1-62; AOCS, 2009), FFA (Ca 5a-40; AOCS, 2009), moisture and volatile matter (Ca 2c-25; AOCS, 2009), insoluble impurities

(Ca 3a-46, AOCS, 2009), unsaponifiable matter (Cb-53, AOCS, 2009), and initial peroxide value (Cd 8b-90; AOCS, 2009). Iodine value was calculated from the fatty acid profile using the following equation: IV = [C16:1] × (0.95) + [C18:1] × (0.86) + [C18:2] × (1.732) + [C18:3] × (2.616) + [C20:1] × (0.795) + [C20:2] × (1.57) + [C20:3] × (2.38) + [C20:4] × (3.19) + [C20:5] × (4.01) + [C22:4] × (2.93) + [C22:5] × (3.68) + [C22:6] × (4.64); brackets indicate percentage concentration (Meadus et al., 2010).

Feed samples were homogenized and then finely ground through a 1 mm screen in a Retsch grinder (model ZMI; Retsch Inc., Newtown, PA). Acid hydrolyzed ether extract (method 2003.06; AOAC, 2007) was determined using a SoxCap SC 247 hydrolyzer and a Soxtec 255 semiautomatic extractor (FOSS North America, Eden Prairie, MN). Dry matter was determined by drying samples in an oven at 105°C to a constant weight. Gross energy was determined using an isoperibolic bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). Benzoic acid (6.318 Mcal/kg; Parr Instrument Co., Moline, IL) was used as the standard for calibration and determined to contain 6.321 ± 0.007 Mcal of GE/kg. All feed analyses were performed in duplicate and repeated when the intra-duplicate CV was greater than 1%.

Gene Abundance

Adipose tissue, liver, and jejunum were homogenized using a PowerGen 700D homogenizer (Fisher Science, Hanover Park, IL). Total RNA was then isolated from the homogenized tissue using TRIzol reagent (Fisher Science, Hanover Park, IL) following the manufacturer's protocol with the modification of repeating the RNA pellet wash step to reduce contaminants. The concentration and quality of RNA was quantified using a spectrophotometer (ND-100, NanoDrop Technologies, Rockland, DE). All samples had 260/280 nm ratios above

1.8 and the integrity of the RNA was further verified by visualization of the 18S and 28S ribosomal bands via a SYBR Safe DNA gel stain (Life Technologies, Carlsbad, CA) after running 2 ug RNA by electrophoresis on a 2% agarose gel. Isolated RNA was then used for cDNA synthesis using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. A spectrophotometer (ND-100, NanoDrop Technologies, Rockland, DE) quantified synthesis of cDNA.

To determine mRNA abundance, quantitative real time PCR was performed using 20 µL reactions prepared according to the manufacturer's instructions using iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA), 1 µL of each forward and reverse primer (diluted with RNAase free H_2O to 100 µ*M*; Table 4.4), and 1 µL of cDNA (diluted 200 ng/µL). Fluorescence of SYBR Green was quantified with a single color MyiQ optical module (Bio-Rad Laboratories, Hercules, CA). Each assay plate contained no-reverse transcriptase negative controls and pooled reference samples. The quantitative real time PCR cycling conditions included a 30 second step at 95°C, and then 38 PCR cycles were run, with each cycle consisting of 3 stages (95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec). Optical detection was performed at 55°C. Analyses of amplification plots were performed with the MyiQ Optical System Software version 1.0 (Bio-Rad Laboratories Inc., Hercules, CA) and cycle threshold (**Ct**) values for each reaction obtained. All mRNA abundance analyses were performed in triplicate and repeated when the intra-triplicate CV was greater than 2%. Expression normalization across samples within tissue was performed by calculating a delta Ct value (**ΔCt =** Ct of the target gene – Ct of the housekeeping gene) for each sample using ribosomal protein-L32 (***RPL32***), as transcript abundance proved to be similar among treatments within tissue ($P = 0.518$). Thus, *RPL32* was considered a suitable housekeeping gene. Delta delta Ct values (**ΔΔCt**) were expressed relative to the CNTR

treatment by the following equation: ΔΔCt = ΔCt of dietary fat treatment – ΔCt of CNTR (Pfaffl, 2001; Duran-Montge et al., 2009). Thus, all ΔΔCt values of CNTR are equal to 1.

Statistical Analysis

The response to the 6 dietary treatments were analyzed using PROC MIXED (SAS 9.4; SAS Inst. Inc., Cary, NC) with treatment as a fixed effect, replicate (2 cohorts of 24 barrows each) as a random effect, and pig as the experimental unit. Determination of the effect of dietary fat regardless of source on mRNA abundance was conducted via a contrast statement between CNTR and the 5 dietary fat treatments (Kaps and Lamberson, 2004; Oehlert, 2010). Determination of the correlation between dietary fatty acid concentration and mRNA abundance was analyzed using PROC CORR (SAS 9.4; Duran-Montge et al., 2009). Non-detectable fatty acid concentrations were treated in all statistical analyses as 0. All *P*-values ≤ 0.05 were considered significant and *P*-values > 0.05 and ≤ 0.10 were considered trends.

Results

Moisture, impurities, and unsaponifiables of the 5 dietary fat sources were ≤ 1.1% (Table 4.2). Analyzed FFA level of the 5 dietary fat sources ranged from 0.08 to 12.80%. Initial peroxide value of FO was 13.8 mEq/kg. The other 4 dietary fat sources had an initial peroxide value of ≤ 1.3 mEq/kg. Fatty acid composition of the 5 sources (Table 4.3) confirmed the selection of sources detailed previously. Thus, the 5 dietary sources were of high quality and provided a diverse array of fatty acid intake.

Due to feed intake being limited, no differences were evident among the 6 dietary fat treatments for feed intake or BW (data not reported; $P \geq 0.753$). In the jejunum, no differences

were evident among the 6 dietary fat treatments for expression of fatty acid binding protein-2 (***FABP-2***) and fatty acid transport protein-4 (***FATP-4***; data not reported; $P \geq 0.175$).

Effects of dietary fat on mRNA abundance in adipose tissue

Pigs fed dietary fat regardless of source decreased fatty acid synthase (***FASN***) gene abundance compared to pigs fed a diet with no added dietary fat ($P = 0.014$; Table 4.5). Of the dietary fat source treatments, pigs fed a COIL-based diet tended to have greater abundance of *FASN* mRNA ($P = 0.071$). Protein kinase, AMP-activated, gamma-1 non-catalytic subunit (***PRKAG-1***) abundance was greater in barrows fed a COIL-based diet than barrows fed CNTR or FO diets ($P = 0.047$). Pigs fed a COIL-based diet increased the abundance of sterol regulatory element-binding protein-1 (***SREBP-1***) more than in pigs fed CNTR or FO diets ($P = 0.025$). There was no impact among the 6 dietary treatments on the mRNA abundance of acetyl CoA carboxylase (***ACACA***), ATP citrate lyase (***ACLY***), adipose triglyceride lipase (***ATGL***), hormone sensitive lipase (***HSL***), peroxisome proliferator activated receptor-alpha (***PPAR-α***), or stearoyl CoA desaturase (***SCD***) ($P \leq 0.125$).

Effects of dietary fat on mRNA abundance in liver

Regardless of source, including fat in the diet had increased abundance of *ACACA*, *ATGL*, *PPAR-α*, *PRKAG-1*, and *SCD* ($P \leq 0.020$; Table 4.6) and tended to have greater abundance of *HSL* ($P = 0.071$) and *SREBP-1* ($P = 0.086$). Hepatic transcription of *ACACA* was greater in pigs fed AV- or TAL-based diets than pigs fed CNTR or FO diets ($P = 0.011$). Pigs fed a TAL-based diet had greater abundance of *ATGL* mRNA than pigs fed CNTR or FO diets ($P = 0.013$). Abundance of *HSL* was greater in pigs fed a TAL-based diet than those fed CNTR,

COCO, or FO diets ($P = 0.013$). Pigs fed a COIL-based diet had greater mRNA abundance of *PPAR-α* than those fed CNTR, COCO, or FO diets ($P < 0.001$). Hepatic *PRKAG-1* transcription was greater in pigs fed AV- or TAL-based diets than pigs fed CNTR, COCO, or FO ($P = 0.004$). Pigs fed AV-, COIL-, or TAL-based diets had greater *SCD* mRNA abundance than those fed CNTR or FO diets ($P = 0.025$). Barrows fed AV-, COIL-, or TAL-based diets tended to have increased mRNA abundance for *ACLY* ($P = 0.098$) and *SREBP-1* ($P = 0.069$) than those fed CNTR, COCO, or FO diets. Abundance of *FASN* tended to be decreased for all pigs fed any of the fat supplemented diets, except for COCO ($P = 0.074$). There was no effect of diet on the abundance of mRNA for fatty acid binding protein-1 (***FABP-1***; $P = 0.914$).

Correlation between dietary fatty acid composition and transcription of genes involved in lipid metabolism

In genes that were affected by dietary treatment ($P \leq 0.050$; Table 4.7), omega-3 concentration was negatively correlated with *SCD* hepatic transcription ($P = 0.042$). Omega-3 concentration also tended to be negatively correlated with adipose tissue *PRKAG*-1 expression ($P = 0.065$) and hepatic *ATGL* expression ($P = 0.063$). Dietary fat source omega-6:omega-3 was positively correlated with adipose tissue *PRKAG-1* abundance ($P = 0.034$). Additionally, omega-6:omega-3 tended to be positively correlated with adipose tissue *SREBP-1* abundance ($P = 0.082$). Dietary fat SFA content was negatively correlated with hepatic transcription of *INSR* and *PPAR-α* ($P \leq 0.039$). Dietary fat MUFA content tended to be positively correlated with hepatic *ACACA*, *PPAR-α*, and *PRKAG-1* mRNA abundance ($P \leq 0.100$). Dietary fat MUFA:SFA was positively correlated with *PPAR-α* abundance in liver ($P = 0.046$).

Additionally, dietary fat MUFA:SFA tended to be positively correlated with *SREBP-1* abundance in adipose tissue ($P = 0.093$).

In genes that tended to be affected by dietary treatment ($P \leq 0.10$; Table 4.8), dietary omega-6 concentration tended to be positively correlated with *FASN* abundance in adipose tissue ($P = 0.085$). Dietary fat SFA concentration was positively correlated with abundance of *FASN* mRNA in liver ($P = 0.050$). Dietary fat MUFA content tended to be positively correlated with hepatic transcription of *ACLY* and *SREBP-1* and negativity correlated with *FASN* abundance ($P \leq 0.100$). Dietary fat PUFA:SFA tended to be positively correlated with *FASN* mRNA abundance in adipose tissue ($P = 0.099$).

Discussion

Changes in adipose tissue lipid metabolism

Unlike in humans or in rodents, de novo lipogenesis primarily occurs in adipose tissue of pigs (O'Hea and Leveille, 1969). The addition of 5% dietary fat decreasing *FASN* abundance compared to CNTR reported herein, supports the generally accepted view that increasing the level of dietary fat suppresses fatty acid synthase function (a multi-faceted enzyme that synthesizes palmitic acid from malonyl CoA in the cytosol of the adipocytes in pigs [Beld et al., 2015]) and reduces the rate of de novo lipogenesis in adipose tissue (Allee et al., 1971; Smith et al., 1996). As a consequence, the fatty acid profile of the carcass reflects that of the diet (Kellner et al., 2014, 2016b). These mRNA abundance data further suggest that the suppression of de novo lipogenesis via mRNA abundance of *FASN* and the transcription factor *SREBP-1* (regulates the expression of key enzymes involved in the lipogenesis pathway [Kim and Spiegleman, 1996; Yahagi et al., 1999]) is reduced when the dietary fat source (i.e. COIL) is high in linoleic acid an

omega-6 fatty acid. Omega-6 fatty acids being a less potent inhibitor of de novo lipogenesis in comparison to other fatty acids is supported in the literature. Duran-Montge et al. (2009) reported a positive correlation between *FASN* mRNA abundance and increased dietary fat omega-6 content and omega-6:omega-3. Kellner et al. (2016a) reported greater mRNA abundance of *FASN* in pigs fed 3% COIL than 3% TAL. Kouba and Mourot (1998) reported greater *ACACA* (a biotin-dependent enzyme which produces malonyl CoA from actetyl CoA in an irreversible reaction which is the rate limiting step of de novo lipogenesis [Volpe and Vagelos, 1976]) and *FASN* expression in COIL-fed pigs than TAL-fed pigs. Smith et al. (1996) observed a greater rate of lipogenesis in cultured porcine adipocytes with a linoleic acid enriched diet versus an oleic acid enriched diet. In contrast, Allee et al. (1971) found that the suppression of lipogenesis was not different in growing pigs fed 10% COIL or TAL.

The observation in adipose tissue that SFAs are a more potent inhibitor of de novo lipogenesis than omega-6 fatty acids (linoleic acid in particular) relates to the fact that dietary fatty acids are largely unmodified in composition (chain length and degree of unsaturation) from ingestion to deposition (Ellis and Isbell, 1926; Kellner et al., 2014). De novo synthesized fatty acids are SFA (i.e. palmitic and stearic acid) or MUFAs (i.e. palmitoleic or oleic acid; Kloareg et al., 2007). Thus, if the pig consumes and deposits SFA of dietary origin, there is less need for the adipocyte to synthesize fatty acids of similar chemical structure (i.e. palmitic, palmitoleic, stearic, or oleic acid). In contrast, if the pig consumes and deposits omega-6 fatty acids (i.e. linoleic acid) the negative feedback on de novo lipogenesis in the adipocyte does not apply to same the degree.

Growing pigs are normally in a positive energy balance; therefore, the pig's reliance on the breakdown of stored lipids via lipolysis for sources of fuel is minimal (Enser, 1984).

However, these data indicate a suppression of *PRKAG-1* (kinase responsible for phosphorylation and activation of proteins involved in the lipolytic cascade such as adipose triglycericde lipase and hormone sensitive lipase [Bijland et al., 2013]) in pigs fed FO (high in omega-3 fatty acids), while all other dietary fat sources increased the mRNA abundance of *PRKAG-1*. Currently there are no porcine *PRKAG-1* abundance data directly to support or contrast this finding. However, supporting evidence of increased omega-3 fatty acid intake causing decreased *PRKAG-1* mRNA and lipolysis rate can be found in experiments with human or rodent subjects. Dietary intake of FO is known to decrease adiposity in rodents fed high fat diets (Belzung et al., 1993; Shearer et al., 2012). Furthermore, rats fed a diet with both FO and TAL versus just TAL decreased plasma NEFA levels and basal intracellular lipolysis by ~50% (Rustan et al., 1993). Intake of omega-3 fatty acids in humans has been shown to suppress protein kinase A in cancer cells in mammary tissue (Moore et al., 2001), and in primary macrophages (Fournier et al., 2016). The suppression of protein kinase A may be due to cell-membrane incorporation of omega-3 fatty acids impairing the upstream signaling pathway to activate protein kinase A (Fournier et al., 2016).

Changes in hepatic lipid metabolism

Though liver is not the primary site of lipogenesis in the pig, it still plays a crucial role in lipid metabolism via lipid transportation, fatty acid oxidation, synthesis of cholesterol and phospholipids, and ketogenesis (Odle et al., 1995). Hepatic lipid metabolism changes were largely correlated to MUFA or SFA content. Dietary fat sources high in MUFA were positively correlated with *ACACA* and tended to be positively correlated with *ACLY* and *SREBP-1*, but were negatively correlated with *FASN*. The explanation of medium chain SFA (C6:0 through C12:0) intake resulting in an increase of *FASN* mRNA abundance in liver and not in adipose

tissue is possibly due to its metabolic endpoint (Foufelle et al., 1992). Medium chain fatty acids once absorbed into the enterocyte enter portal capillaries and are transported via the portal vain to the liver (Odle, 1997). This is in contrast to longer chain SFA, MUFA, and PUFA which are packaged into chylomicrons, directed through the lymphatic system, and then circulated to target peripheral tissues (i.e. adipose and muscle; Bach and Babayan, 1982; Odle, 1997). Thus, the exposure of digested and absorbed medium chain fatty acids from COCO is greater in liver than adipose tissue (Foufelle, 1992; Odle, 1997).

Duran-Montge et al. (2009) reported in growing pigs a similar positive correlation between dietary MUFA concentration and mRNA abundance of *ACACA* and *SREBP-1*, but found no significant correlation between dietary MUFA concentration and mRNA abundance of *FASN*. The differences between the Duran-Montge et al. (2009) correlations and the correlations reported herein, may be due to chain length and degree of saturation levels of dietary fat (TAL vs. COCO).

In continuation of medium chain fatty acids being directed to the liver versus peripheral tissues, hepatic expression of *PPAR-α* (transcription factor of fatty acid oxidation [Lee et al., 1995; Duran-Montge et al., 2009]), and *PRKAG-1* was also decreased in pigs fed COCO. These decreases of mRNA abundance in COCO-fed pigs may explain the positive correlations between MUFA content and *PPAR-α* and *PRKAG-1* and negative correlations with SFA *PPAR-α* mRNA abundances.

In animals where hepatic lipogenesis occurs at a greater proportion than found in pigs. Hepatic transcription of *FASN* was increased in rainbow trout fed 5% COCO compared to 5% FO (Figueiredo-Silva et al., 2011). Hepatic transcription of *FASN* was also increased in rats fed 33% palm oil (a fat source comprised mainly of medium chain length, SFAs; NRC, 2012) versus

rats fed 32% COIL (Foufelle et al., 1992). Foufelle et al. (1992) also found that mRNA abundance of *ACACA* was higher in palm oil versus COIL.

As reported in adipose tissue, omega-3 fatty acid intake decreased the transcription of genes related to lipolysis in the liver. In comparison to the other dietary fat treatments, pigs fed FO had reduced mRNA abundance of *PRKAG-1*, *ATGL*, and *HSL*. Omega-3 fatty acids are known to lower plasma triglycerides and non-esterified fatty acid levels (Rustan et al., 1993; Shearer et al., 2012). This is due to omega-3 fatty acids from FO increasing the activity of lipoprotein lipase in adipose and muscle and stimulating β-oxidation in muscle (Shearer et al., 2012). Thus, in comparison to the other long chained dietary fat sources tested in this experiment, FO could reduce fatty acids being delivered to the liver. This may explain why the transcription of genes involved in hepatic lipolysis were decreased. This explanation is supported by Rustan et al. (1993) who reported reduced whole body lipid utilization in rats fed omega-3 fatty acids compared with lard. In contrast, Sun et al. (2011) reported an increase in hepatic lipolysis and expression of *HSL* in mice fed increasing amounts of docosahexaenoic acid (omega-3 fatty acid).

These mRNA abundances could possibly be decreased by omega-3 incorporation into cellular membrane phospholipids, causing a disruption of membrane protein function and resulting in a suppression of the lipolytic cascade (Fournier et al., 2016). Clearly, more work is needed in determining the impact of omega-3 fatty acids on the rate of lipid breakdown and porcine hepatic tissue.

Conclusion

Added dietary fat will generally suppress the expression of genes involved in lipogenesis and increase the expression of lipolysis related genes. Intake of omega-3 fatty acids suppresses the transcription of genes involved in lipolysis in both adipose tissue and liver. Dietary SFA are more potent inhibitors than omega-6 fatty acids of the transcription of genes involved in de novo lipogenesis in adipose tissue. Due to their metabolic endpoint in the liver versus peripheral tissues, medium chain fatty acids have different effects than longer chain fatty acids on hepatic transcription of lipid metabolism genes.

Literature cited

Allee, G. L., D. H. Baker, and G. A. Leveille. 1971. Influence of dietary fat on adipose tissue lipogenesis and enzymatic activity in the pig. J. Anim. Sci. 33:1248-1254. doi:10.2527/jas1971.3361248x.

American Oil Chemists' Society (AOCS). 2009. Recommended practice Ca 3a-46, Ca 5a-40, Ca 2c-25, Cb-53, Ce 1-62, Cd 8b-90. In Official Methods and Recommended Practices of the AOCS. 6th ed. AOCS, Urbana, IL.

Bach, A. C. and V. K. Babayan. 1982. Medium-chain triglycerides: an update. Am. J. Clin. Nutr. 36:950-962.

Beld, J., D. J. Lee, and M. D. Burkart. 2015. Fatty acid biosynthesis revisited: structure elucidation and metabolic enginerring. Mol. Biosyst. 11:38-59. doi:10.1039/c4mb00443d.

Bergen, W. G., and H. J. Mersmann. 2005. Comparative aspects of lipid metabolism: impact on contemporary research and use of animal models. J. Nutr. 135:2499-2502.

Belzung, F., T. Raclot, and R. Groscolas. 1993. Fish oil n-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets. Am. J. Physiol. 264:1111-1118.

Bijland, S., S. J. Mancini, and I. P. Salt. 2013. Role of AMP-activated protein kinase in adipose tissue metabolism and inflammation. Clin. Sci. (Lond). 124:491-507. doi:10.1042/CS20120536.

Bortz, W., S. Abraham, and I. L. Chaikoff. 1963. Localization of the block in lipogenesis resulting from feeding fat. J. Biol. Chem. 238:1266-1276.

Duran-Montge, P., P. K. Theil, C. Lauridsen, and E. Esteve-Garcia. 2009. Dietary fat source affects metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. Animal. 3:535-542. doi:10.1017/S1751731108003686.

Ellis, N. R., and H. S. Isbell. 1926. Soft Pork Studies. II. The influence of the character of the ration upon the composition of the body fat of hogs. J. Biol. Chem. 59:219-248.

Enser, M. 1984. The chemistry, biochemistry and nutritional importance of animal fats. In: J. Wiseman, editor, Fats in animal nutrition. Butterworths, London, UK. P. 23-51.

Figueiredo-Silva, A. C., S. Kaushik, F. Terrier, J. W. Schrama, F. Medale, and I. Geurden. 2012. Link between lipid metabolism and voluntary food intake in rainbow trout fed coconut oil rich in medium-chain TAG. Br. J. Nutr. 107:1714-1725. doi:10.1017/S0007114511004739.

Foufelle, F., D. Perdereau, B. Gouhot, P. Ferre, and J. Girard. 1992. Effect of diets rich in medium-chain and long-chain triglycerides on lipogenic-enzyme gene expression in liver and adipose tissue of the weaned rat. Eur. J. Biochem. 208:381-387.

Fournier, N., S. Tardivel, J. F. Benoist, B. Vedie, D. Rousseau-Ralliard, M. Nowak, F. Allaoui, and J. L. Paul. 2016. Eicosapentaenoic acid membrane incorporation impairs ABCA1-dependent cholesterol efflux via a protein kinase A signaling pathway in primary human macrophages. Biochim. Biophys. Acta. 1861:331-341. doi:10.1016/j.bbalip.2016.01.002.

Gaidhu, M. P., S. Fediuc, N. M. Anthony, M. So, M. Mirpourian, R. L. Perry, and R. B. Ceddia. 2009. Prolonged AICAR-induced AMP-kinase activation promotes energy dissipation in white adipocytes: novel mechanisms integrating HSL and ATGL. J. Lipid. Res. 50:704-715. doi:10.1194/jlr.M800480-JLR200.

Hsu, J. M., P. H. Wang, B. H. Liu, and S. T. Ding. 2004. The effect of dietary docosahexaenoic acid on the expression of porcine lipid metabolism-related genes. J. Anim. Sci. 82:683-689. doi:10.2527/2004.823683x.

Jump, D. B. 2002. Dietary polyunsaturated fatty acids and regulation of gene transcription. Curr. Opin. Lipidol. 13:155-164.

Kaps, M., and W. R. Lamberson. 2004. One-way analysis of variance. In: M. Kaps and W. R. Lamberson, editors, Biostatistics for animal science. CABI Publishing, Cambridge, Massachusetts. p. 220-228.

Kellner, T. A., L. H. Baumgard, K. J. Prusa, N. K. Gabler, and J. F. Patience. 2016a. Does heat stress alter the pig's response to dietary fat? J. Anim. Sci. 94:4688-4703. doi:10.2527/jas.2016-0756.

Kellner, T. A., G. G. Gourley, S. Wisdom, and J. F. Patience. 2016b. Prediction of porcine carcass iodine value based on diet composition and fatty acid intake. J. Anim. Sci. 94:5248-5261. doi:10.2527/jas.2016-0643.

Kellner, T. A., K. J. Prusa, and J. F. Patience. 2015. Impact of dietary fat withdrawal on carcass iodine value, belly characteristics, and changes in body fat over time. J. Anim. Sci. 93:247-257. doi:10.2527/jas.2014-7650.

Kellner, T. A., K. J. Prusa, and J. F. Patience. 2014. Impact of dietary fat source and concentration and daily fatty acid intake on the composition of carcass fat and iodine value sampled in three regions of the pork carcass. J. Anim. Sci. 92:5485-5495 doi:10.2527/jas.2014-7567.

Kim, J. B., and B. M. Spiegelman. 1996. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev. 10:1096-1107.

Kloareg, M., J. Noblet, and J. van Milgen. 2007. Deposition of dietary fatty acids, de novo synthesis and anatomical partitioning of fatty acids in finishing pigs. Br. J. Nutr. 97:35–44. doi:10.1017/S0007114507205793.

Kouba, M., and J. Mourot. 1998. Effect of a high linoleic acid diet on delta 9-desaturase activity, lipogenesis and lipid composition of pig subcutaneous adipose tissue. Reprod. Nutr. Dev. 38:31-37.

Lee, S. S., T. Pineau, J. Drago, E. J. Lee, J. W. Owens, D. L. Kroetz, P. M. Fernandez-Salguero, H. Westphal, and F. J. Gonzalez. 1995. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol. Cell. Biol. 15:3012-3022. doi:10.1128/MCB.15.6.3012.

Lee, J. H., J. Kong, J. Y. Jang, J. S. Han, Y. Ji, J. Lee, and J. B. Kim. 2014. Lipid droplet protein LID-1 mediates ATGL-1-dependent lipolysis during fasting in Caenorhabditis elegans. 34:4165-4176. doi:10.1128/MCB.00722-14.

Meadus, W. J., P. Duff, B. Uttaro, J. L. Aalhus, D. C. Rolland, L. L. Gibson, and M. E. Dugan. 2010. Production of docosahexaenoic acid (DHA) enriched bacon. J. Agric. Food Chem. 58:465-472. doi:10.1021/jf9028078.

Moore, N. G., F. Wang-Johanning, P. L. Chang, and G. L. Johanning. 2001. Omega-3 fatty acids decrease protein kinase expression in human breast cancer cells. Breast Cancer Res. Treat. 67:279-283.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Odle, J. 1997. New insights into the utilization of medium-chain triglycerides by the neonate: observations form a piglet model. J. Nutr. 127:1061-1067.

Odle, J., X. Lin, T. A. van Kempen, J. K. Drackley, and S. H. Adams. 1995. Carnitine palmitoyltransferase modulation of hepatic fatty acid metabolism and radio-HPLC evidence for low ketogenesis in neonatal pigs. J. Nutr. 125:2541-2549.

Oehlart, G. W. 2010. Looking for specific differences – contrasts. In: G. W. Oehlart, editor, A first course in design and analysis of experiments. W. H. Freeman and Company, San Franscico, Califorina. p. 67.

O'Hea, E. K., and G. A. Leveille. 1969. Significance of adipose tissue and liver as sites of fatty acid synthesis in the pig and the efficiency of utilization of various substrates for lipogenesis. J. Nutr. 99:338-344.

Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29:2002-2007.

Powles, J., J. Wiseman, D. J. A. Cole, and S. Jagger. 1995. Prediction of the apparent digestible energy value of fats given to pigs. Anim. Sci. 61:149-154. doi:1357-7298/95/45810149S20.00111.

Rustan, A. C., B. E. Hustvedt, and C. A. Drevon. 1993. Dietary supplementation of very long-chain n-3 fatty acids decreases whole body lipid utilization in the rat. J. Lipid Res. 34:1299-1309.

Shearer, G. C., O. V. Savinova, and W. S. Harris. 2012. Fish oil – how does it reduce plasma triglycerides? Biochim. Biophys. Acta. 1821:843-851. doi:10.1016/j.bbalip.2011.10.011.

Smith, D. R., D. A. Knabe, and S. B. Smith. 1996. Depression of lipogenesis in swine adipose tissue by specific dietary fatty acids. J. Anim. Sci. 74:975-983. doi:10.2527/1996.745975x.

Sun, C., Z. Wei, and Y. Li. 2011. DHA regulates lipogenesis and lipolysis genes in mice adipose and liver. Mol. Biol. Rep. 38:731-737. doi:10.1007/s11033-010-0160-9.

Volpe, J. J., and P. R. Vagelos. 1976. Mechanisms and regulation of biosynthesis of saturated fatty acids. Physiol. Rev. 56:339-417.

Yahagi, N., H. Shimano, A. H. Hasty, M. Amemiya-Kudo, H. Okazaki, Y. Tamura, Y. Iizuka, F. Shionoiri, K. Ohashi, J. Osuga, K. Harada, T. Gotoda, R. Nagai, S. Ishibashi, and N. Yamada. A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J. Biol. Chem. 274:35840-35844. doi:10.1074/jbc.274.50.35840.

Table 4.1. Ingredient and nutrient composition (as-fed basis) of experimental diets d 0 to 10

Item	Control	Animal-vegetable blend	Coconut oil	Corn oil	Fish oil	Tallow
Ingredient, %						
Corn	68.41	68.41	68.41	68.41	68.41	68.41
Soybean meal (46.5% CP)	22.50	22.50	22.50	22.50	22.50	22.50
Corn starch	5.00	-	-	-	-	-
Experimental dietary fat	-	5.00	5.00	5.00	5.00	5.00
Limestone	0.96	0.96	0.96	0.96	0.96	0.96
Monocalcium phosphate (21%)	1.22	1.22	1.22	1.22	1.22	1.22
Salt	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine HCL	0.33	0.33	0.33	0.33	0.33	0.33
DL-methionine	0.10	0.10	0.10	0.10	0.10	0.10
L-threonine	0.12	0.12	0.12	0.12	0.12	0.12
Trace mineral premix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[2]	0.20	0.20	0.20	0.20	0.20	0.20
Santoquin[3]	0.06	0.06	0.06	0.06	0.06	0.06
Titainium dioxide	0.40	0.40	0.40	0.40	0.40	0.40
Analyzed composition						
DM, %	86.66	87.35	87.77	86.79	87.61	87.45
GE, Mcal/kg	3.89	4.07	4.06	4.05	4.06	4.09
Acid hydrolyzed ether extract, %	2.97	9.32	8.94	8.55	9.14	9.21

[1]Provided 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kilogram of diet.

[2]Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.023 mg vitamin B12 per kilogram of diet.

[3]Santoquin Mixture 6 (feed and forage antioxidant; Novus International, St. Charles, MO).

Table 4.2. Analyzed chemical composition of dietary fat sources[1]

Item	Animal-vegetable blend[2]	Coconut oil[3]	Corn oil[4]	Fish oil[5]	Tallow[6]
Free fatty acid, %	7.00	0.08	12.80	2.80	3.60
Moisture and volatile matter, %	0.06	0.02	0.42	0.34	0.06
Insoluble impurities, %	0.02	0.02	0.02	0.06	0.06
Unsaponifiable matter, %	0.41	0.23	0.47	0.69	0.31
MIU,[7] %	0.49	0.27	0.91	1.09	0.43
Initial peroxide value, mEq/kg	0.30	0.20	0.60	13.80	1.30

[1]Analysis via Barrow Agee Laboratories (Memphis, TN).
[2]Sourced via Darling Pro Ingredients (Wahoo, NE).
[3]Sourced via Bulk Apothecary (Aurora, OH).
[4]Sourced via Feed Energy Co. (Des Moines, IA).
[5]Sourced via Double S Liquid Feed Serves, Inc. (Danville, IL).
[6]Sourced via Darling Pro Ingredients (Omaha, NE).
[7]MIU = moisture, impurities, and unsaponifiables.

Table 4.3. Analyzed fatty acid composition of dietary fat sources[1]

Item	Animal-vegetable blend[2]	Coconut oil[3]	Corn oil[4]	Fish oil[5]	Tallow[6]
Fatty acid, %					
Caprylic acid (C8:0)	ND[9]	6.17	ND	ND	ND
Capric acid (C10:0)	ND	5.39	ND	ND	ND
Lauric acid (C12:0)	ND	48.46	ND	0.11	ND
Myrsitic acid (C14:0)	1.63	19.75	ND	9.88	2.78
Palmitc acid (C16:0)	22.39	9.44	11.92	20.33	24.08
Palmitoleic acid (C16:1 n-9)	2.92	ND	0.09	11.66	2.48
Hexadecadienoic acid (C16:2 n-4)	ND	ND	ND	ND	1.43
Margaric acid (C17:0)	0.46	ND	ND	0.82	1.22
Stearic acid (C18:0)	10.45	9.08	1.71	3.49	20.29
Oleic acid (C18:1 n-9)	45.25	1.07	27.20	9.28	41.59
Linoleic acid (C18:2 n-6)	13.41	0.06	56.84	1.15	2.81
Linolenic acid (C18:3 n-3)	0.62	ND	1.35	1.34	0.31
Octadecatetraenoic acid (C18:4 n-3)	ND	ND	ND	2.01	ND
Arachidonic acid (C20:4 n-6)	0.24	ND	ND	1.36	ND
Eicosapentaenoic acid (C20:5 n-3)	ND	ND	ND	14.32	ND
Docosapentaenoic (C22:5 n-3)	ND	ND	0.16	2.81	ND
Docosahexaenoic acid (C22:6 n-3)	ND	ND	ND	8.22	ND
Other fatty acids	2.61	0.58	0.75	11.78	4.44
Omega-3, %	0.62	0.00	1.51	29.08	0.31
Omega-6, %	14.22	0.06	56.84	2.94	2.81
Omega-6/Omega-3	22.94	NC[10]	37.64	0.10	9.06
MUFA, %	49.46	1.07	27.55	22.82	44.95
PUFA, %	14.84	0.06	58.35	33.85	3.12
SFA, %	35.22	98.87	14.12	35.76	48.92
MUFA/PUFA	3.33	17.83	0.47	0.67	14.41
MUFA/SFA	1.40	0.01	1.95	0.64	0.92
PUFA/SFA	0.42	0.00	4.13	0.95	0.06
Iodine value[7]	68.7	1.0	126.3	137.4	44.0
U:S[8]	1.83	0.01	6.08	1.58	0.98

[1]Analysis via Barrow Agee Laboratories (Memphis, TN).

[2]Sourced via Darling Pro Ingredients (Wahoo, NE).

[3]Sourced via Bulk Apothecary (Aurora, OH).

[4]Sourced via Feed Energy Co. (Des Moines, IA).

[5]Sourced via Double S Liquid Feed Serves, Inc. (Danville, IL).

[6]Sourced via Darling Pro Ingredients (Omaha, NE).

[7]Iodine value calculated from fatty acid composition: IV = [C16:1] × (0.95) + [C18:1] × (0.86) + [C18:2] × (1.732) + [C18:3] × (2.616) + [C20:1] × (0.795) + [C20:2] × (1.57) + [C20:3] × (2.38) + [C20:4] × (3.19) + [C20:5] × (4.01) + [C22:4] × (2.93) + [C22:5] × (3.68) + [C22:6] × (4.64); brackets indicate percentage concentration (Meadus et al., 2010).

[8]Unsaturated to saturated fatty acid ratio.

[9]Non-dectable.

[10]Non-calcuable.

Table 4.4. Forward and reverse primer sequences

Gene	Description	Forward primer 5'-3'	Reverse primer 5'-3'
ACACA	Acetyl CoA carboxylase	ATGGATGAACCGTCTCCC	TGTAAGGCCAAGCCATCC
ACLY	ATP citrate lyase	AGGAGGAGTTCTATGTCTGC	CAACAGGTGTTTCTTGATGGCC
ATGL (PNPLA2)	Adipose triglyceride lipase (Patatin-like phospholipase domain containing 2)	ATCATAACCCACTTCGCC	ACACGGGAATGAAGGTGC
FABP-1	Fatty acid binding protein 1	ACATCAAGGGGACATCGG	GTCTCCATCTCACACTCC
FABP-2	Fatty acid binding protein 2	GGTAAAGAGGAAACTTGC	AGTGAGTTCAGTTCCGTCTGC
FATP-4	Fatty acid transport protein 4	AGCTCTTCTACATCTACACG	AATCCGTAGTACACCAGG
FASN	Fatty acid synthase	CACAACTCCAAAGACACG	AGGAACTCGGACATAGCG
HSL	Hormone sensitive lipase	AACGCAATGAAACAGGCC	TGTATGATCCGCTCAACTCG
PPAR-α	Peroxisome proliferator activated receptor-alpha	AACGGCATCCAGAACAAG	CATCACAGAGGACAGCATGG
PRKAG-1	Protein kinase, AMP-activated, gamma 1 non-catalytic subunit	TTGGTGACTAATGGTGTCCG	TGAAATCAGTGATGGTCAGC
SCD	Stearoyl CoA desaturase	TACTATCTGCTGAGTGCTGTGG	CTGGAATGCCATCGTGTTGG
SREBP-1	Sterol regulatory element-binding protein 1	TGGCGCTTCTCTTTGTCTATGG	GTGCTAGAGAGTCAGTGG

Table 4.5. Effects of dietary fat source on mRNA abundance in adipose tissue[1]

		Dietary treatment[2], ΔΔCt[3]							*P*-value	
Gene	Description	CNTR	AV	COCO	COIL	FO	TAL	SEM	TRT[4]	CNTR vs. DF[5]
ACACA	Acetyl CoA carboxylase	1.00	1.00	1.74	2.59	-0.33	0.32	0.91	0.249	0.946
ACLY	ATP citrate lyase	1.00	1.94	1.27	2.74	-0.14	0.80	0.98	0.422	0.764
ATGL (PNPLA2)	Adipose triglyceride lipase (Patatin-like phospholipase domain containing 2)	1.00	1.81	2.45	3.97	0.20	3.14	1.00	0.125	0.236
FASN	Fatty acid synthase	1.00	-1.37	-0.74	0.25	-1.44	-0.99	1.25	0.071	0.014
HSL	Hormone sensitive lipase	1.00	2.86	2.99	2.46	0.47	2.98	1.07	0.223	0.186
PPAR-α	Peroxisome proliferator activated receptor-alpha	1.00	3.27	2.10	3.57	2.27	2.38	1.13	0.635	0.163
PRKAG-1	Protein kinase, AMP-activated, gamma 1 non-catalytic subunit	1.00^{bc}	2.72^{ab}	3.08^{ab}	4.00^{a}	-0.22^{c}	1.80^{abc}	0.97	0.047	0.235
SCD	Stearoyl CoA desaturase	1.00	0.20	0.61	1.43	-0.23	-0.12	1.06	0.797	0.552
SREBP-1	Sterol regulatory element-binding protein 1	1.00^{c}	3.78^{a}	1.29^{bc}	3.65^{ab}	0.45^{c}	1.51^{abc}	0.83	0.025	0.219

[a,b,c]Within a row, least square means lacking a common superscript differ ($P < 0.05$).

[1]Total of 48 barrows (8 per treatment) with an initial BW of 44.1 ± 1.2 kg and a final (d 10) BW of 51.2 ± 1.7 kg.

[2]Each experimental diet included 95% of a corn-soybean meal basal diet and then 5% of either: corn starch (control; CNTR), animal-vegetable blend (AV), coconut oil (COCO), corn oil (COIL), fish oil (FO), or tallow (TAL).

[3]Delta delta cycle threshold (Ct).

[4]Probability value of obtaining the observed difference among the 6 dietary treatments.

[5]Probability value of obtaining the observed difference between CNTR and 5 dietary fat (DF) treatments.

Table 4.6. Effects of dietary fat source on mRNA abundance in liver[1]

		Dietary treatment[2], ΔΔCt[3]							*P*-value	
Gene	Description	CNTR	AV	COCO	COIL	FO	TAL	SEM	TRT[4]	CNTR vs. DF[5]
ACACA	Acetyl CoA carboxylase	1.00^{c}	5.12^{a}	2.90^{abc}	3.96^{ab}	2.09^{bc}	5.15^{a}	1.05	0.011	0.006
ACLY	ATP citrate lyase	1.00	3.67	1.13	3.03	0.47	3.80	1.05	0.098	0.222
ATGL (PNPLA2)	Adipose triglyceride lipase (Patatin-like phospholipase domain containing 2)	1.00^{c}	4.05^{ab}	3.59^{ab}	4.37^{ab}	2.36^{bc}	4.92^{a}	1.11	0.013	0.002
FABP-1	Fatty acid binding protein 1	1.00	1.06	1.01	0.39	0.56	0.20	1.92	0.914	0.455
FASN	Fatty acid synthase	1.00	-2.01	2.64	-0.87	-1.49	-0.78	2.00	0.074	0.255
HSL	Hormone sensitive lipase	1.00^{b}	2.31^{ab}	1.37^{b}	2.30^{ab}	0.93^{b}	3.36^{a}	0.52	0.013	0.071
PPAR-α	Peroxisome proliferator activated receptor-alpha	1.00^{c}	7.33^{ab}	1.78^{c}	8.54^{a}	5.67^{b}	7.93^{ab}	0.89	<0.001	<0.001
PRKAG-1	Protein kinase, AMP-activated, gamma 1 non-catalytic subunit	1.00^{b}	4.00^{a}	1.94^{b}	2.70^{ab}	1.34^{b}	3.95^{a}	0.87	0.004	0.014
SCD	Stearoyl CoA desaturase	1.00^{b}	4.21^{a}	2.76^{ab}	3.92^{a}	1.09^{b}	3.94^{a}	1.16	0.025	0.020
SREBP-1	Sterol regulatory element-binding protein 1	1.00	4.33	1.82	3.07	1.33	3.68	0.98	0.069	0.086

[a,b,c]Within a row, least square means lacking a common superscript differ ($P < 0.05$).

[1]Total of 48 barrows (8 per treatment) with an initial BW of 44.1 ± 1.2 kg and a final BW (d 10) of 51.2 ± 1.7 kg.

[2]Each experimental diet included 95% of a corn-soybean meal basal diet and then 5% of either: corn starch (control; CNTR), animal-vegetable blend (AV), coconut oil (COCO), corn oil (COIL), fish oil (FO), or tallow (TAL).

[3]Delta delta cycle threshold (Ct).

[4]Probability value of obtaining the observed difference among the 6 dietary treatments.

[5]Probability value of obtaining the observed difference between CNTR and 5 dietary fat (DF) treatments.

Table 4.7. Correlation coefficients (*r*) between dietary fatty acid composition and mRNA abundance of genes that were affected by dietary fat treatment in adipose (AT) and liver (LT)

	Gene[1]							
	PRKAG-1	*SREBP-1*		*ATGL*	*HSL*		*PRKAG-1*	*SCD*
Item	AT	AT	*ACACA* LT	LT	LT	*PPAR-α* LT	LT	LT
Omega-3	-0.885*	NS[2]	NS	-0.859*	NS	NS	NS	-0.891**
Omega-6	NS	NS	NS	NS	NS	NS	NS	NS
Omega-6/Omega-3	0.966**	0.918*	NS	NS	NS	NS	NS	NS
SFA	NS	NS	NS	NS	NS	-0.898**	NS	NS
MUFA	NS	NS	0.803*	NS	NS	0.828*	0.829*	NS
PUFA	NS	NS	NS	NS	NS	NS	NS	NS
MUFA/PUFA	NS	NS	NS	NS	NS	NS	NS	NS
MUFA/SFA	NS	0.815*	NS	NS	NS	0.885**	NS	NS
PUFA/SFA	NS	NS	NS	NS	NS	NS	NS	NS
U:S	NS	NS	NS	NS	NS	NS	NS	NS
Iodine Value	NS	NS	NS	NS	NS	NS	NS	NS

*Probability value of obtaining the observed coefficient ($P \leq 0.100 \geq 0.051$).

**Probability value of obtaining the observed coefficient ($P \leq 0.050$).

[1]Description of genes: *ACACA*: acetyl CoA carboxylase, *ATGL*: adipose triglyceride lipase (*PNPLA2* [Patatin-like phospholipase domain containing 2]), *HSL*: hormone sensitive lipase, *PPAR-α* peroxisome proliferator activated receptor-alpha, *PRKAG-1*: protein kinase, AMP-activated, gamma 1 non-catalytic subunit, *SCD*: stearoyl CoA desaturase, *SREBP-1*: sterol regulatory element-binding protein 1.

[2]Non-significant ($P \geq 0.101$).

Table 4.8. Correlation coefficients (*r*) between dietary fatty acid composition and mRNA abundance of genes that tended to be affected by dietary fat treatment in adipose (AT) and liver (LT)

	Gene			
	FASN	*ACLY*	*FASN*	*SREBP-1*
Item	AT	LT	LT	LT
Omega-3	NS[2]	NS	NS	NS
Omega-6	0.826*	NS	NS	NS
Omega-6/Omega-3	NS	NS	NS	NS
SFA	NS	NS	0.876**	NS
MUFA	NS	0.804*	-0.825*	0.839*
PUFA	NS	NS	NS	NS
MUFA/PUFA	NS	NS	NS	NS
MUFA/SFA	NS	NS	NS	NS
PUFA/SFA	0.806*	NS	NS	NS
U:S	NS	NS	NS	NS
Iodine Value	NS	NS	NS	NS

*Probability value of obtaining the observed coefficient ($P \leq 0.100 \geq 0.051$).

**Probability value of obtaining the observed coefficient ($P \leq 0.050$).

[1]Description of genes: *ACLY*: ATP citrate lyase, FASN: fatty acid synthase, *SREBP-1*: sterol regulatory element-binding protein 1.

[2]Non-significant ($P \geq 0.101$).

CHAPTER V

THE DEFINITION AND THE EXPLANATION OF THE DE, ME AND NE CONTENT OF DIETARY FAT SOURCES IN 13 AND 50 KG PIGS

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T. A. Kellner* and J. F. Patience*

*Department of Animal Science, Iowa State University, Ames, 50011, USA

Abstract

The objective was to determine the energy concentration for a diverse array of dietary fat sources and from these data, develop regression equations that explain differences based on chemical composition. A total of 120 Genetiporc 6.0 × Genetiporc F25 (PIC, Inc., Hendersonville, TN) individually housed barrows were studied for 56 d. These barrows (initial BW of 9.9 ± 0.6 kg) were randomly allotted to 1 of 15 dietary treatments. Each experimental diet included 95% of a corn-soybean meal basal diet plus 5% either: corn starch or 1 of 14 dietary fat sources. The 14 dietary fat sources (animal-vegetable blend, canola oil, choice white grease source A, choice white grease source B, coconut oil, corn oil source A, corn oil source B, fish oil, flaxseed oil, palm oil, poultry fat, soybean oil source A, soybean oil source B, and tallow) were selected to provide a diverse and robust range of U:S (unsaturated fatty acid:SFA). Pigs were limit-fed experimental diets from d 0 to 10 and d 46 to 56 providing a 7 d adaption for fecal collection on d 7 to 10 (13 kg BW) and d 53 to 56 (50 kg BW). At 13 kg BW, the average energy content of

the 14 sources was 8.42 Mcal of DE/kg, 8.26 Mcal of ME/kg, and 7.27 Mcal of NE/kg, respectively. At 50 kg BW, the average energy content was 8.45 Mcal of DE/kg, 8.28 Mcal of ME/kg, and 7.29 Mcal of NE/kg, respectively. At 13 kg BW, variation of dietary fat DE content was explained by: DE (Mcal/kg) = 9.363 + [0.097 × (FFA, %)] – [0.016 × Omega-6:Omega-3] – [1.240 × (arachidic acid, %)] – [5.054 × (insoluble impurities, %)] + [0.014 × (palmitic acid, %)] (P = 0.008; R^2 = 0.82). At 50 kg BW, variation of dietary fat DE content was explained by: DE (Mcal/kg) = 8.357 + [0.189 × U:S] – [0.195 × (FFA, %)] – [6.768 × (behenic acid, %)] + [0.024 × (PUFA, %)] (P = 0.002; R^2 = 0.81). In summary, the chemical composition of dietary fat explained a large degree of the variation observed in the energy content of dietary fat sources. The Powles et al. (1995) equation accurately predicted the average DE content from the 14 sources (8.43 Mcal/kg), but underestimated the DE content of medium chain SFA sources and the negative impact of increased FFA level to a large degree. Further research is needed to validate if the equations generated herein are more precise in predicting dietary fat DE variation among sources.

Introduction

Fat is included in swine diets as a source of energy when the cost is economically advantageous. However, DE, ME and NE content estimates of dietary fat have been variable and have not been fully validated in commercial conditions (Kil et al., 2011; Boyd et al., 2015). A lack of precision in defining the energy value of dietary fat could lead to losses for pork producers due to incorrect costing in diet formulations and disappointing performance outcomes.

Prediction equations compiled by Powles et al. (1995) using data from Wiseman et al. (1990) and Powles et al. (1993, 1994) have been commonly used to estimate the energy content

of fat sources by using the unsaturated fatty acid to SFA ratio (**U:S**) and FFA level. The ME and NE content is then often estimated from DE according to van Milgen et al. (2001) who suggested that ME is 98% of DE and NE is 88% of ME. The NRC (2012) points out that the equation accuracy across all compositions and characteristics of dietary fat sources is unknown. Boyd et al. (2015) recently utilized a growth assay to determine the NE content of choice white grease and reported a 14% difference compared to the NRC (2012) estimate. Clearly, validation and refinement of the energy values assigned to dietary fat sources in swine is needed. Including dietary fatty acid concentration and more detailed chemical composition along with FFA and U:S content across a diverse and robust range of dietary fat sources may generate a more accurate estimate of the DE, ME and NE of dietary fat.

Thus, the objective was to determine the energy concentration in a diverse array of dietary fat sources and from these data, develop regression equations that explain differences based on chemical composition, and thus could serve as prediction equations in the future. The hypothesis was that dietary fat DE variation among sources can be more accurately explained using a more detailed chemical composition than previous attempts.

Materials and methods

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research, and were approved by the Iowa State University Institutional Animal Care and Use Committee (#2-16-8201-S).

Animals, Housing, and Experimental Design

A total of 120 Genetiporc 6.0 × Genetiporc F25 (PIC, Inc., Hendersonville, TN) barrows in 2 sequential replicate groups of 60 barrows each were studied. These barrows (initial BW of 9.9 ± 0.6 kg) were allotted at random to 1 of 15 dietary treatments: (control [**CNTR**], animal-vegetable blend [**AV**], canola oil [**CANO**], choice white grease source A [**CWGA**], choice white grease source B [**CWGB**], coconut oil [**COCO**], corn oil source A [**CORA**], corn oil source B [**CORB**], fish oil [**FISH**], flaxseed oil [**FLAX**], palm oil [**PALM**], poultry fat [**POUF**], soybean oil source A [**SOYA**], soybean oil source B [**SOYB**], and tallow [**TAL**]).

Pigs were housed individually throughout the 56 d experiment. From d 0 to 28 pigs were housed in a room in which each pen provided 0.50 m^2 of floor space per pig, a nipple drinker, and a stainless steel feeder and had mesh metal flooring. From d 28 to 56 pigs were housed in a room in which each pen provided 1.83 m^2 of floor space per pig, a nipple drinker, and a composite feeder and had slatted concrete flooring.

Diets and Feeding

Each experimental diet (Table 5.1 and 5.2) included 95% of a corn-soybean meal basal diet plus 5% of either: corn starch (CNTR) or 1 of the previously listed 14 dietary fat sources. Pigs were fed their assigned diets from d 0 to 10 (Table 5.1) and d 46 to 56 (Table 5.2). These experimental periods provided a 7 d acclimation to the diet prior to fecal collection. Pigs were fed the same fat source in both experimental periods and fed a common diet between experimental periods (d 10 to 46; Table 5.3). Feed allowance was limited from d 0 to 10 to provide a daily energy intake of 2.8 times maintenance (NRC, 2012). From d 10 to 46 feed was provided ad libitum. Feed allowance was limited from d 46 to 56 to provide a daily energy

intake of 3.2 times maintenance (NRC, 2012). Feed allowances were selected for each phase to maximize intake without having variation of feed intake among pigs. Water was provided *ad libitum* at all times from d 0 to 56. Dietary fat sources were selected to provide a diverse range of degree of unsaturation. The chemical composition and the fatty acid profile of the sources of dietary fat are presented in Tables 5.4, 5.5 and 5.6, respectively.

Representative feed samples were collected at the time of mixing and stored at -20°C for later analysis. Representative dietary fat samples were collected by subsampling from a minimum of 5 different locations. The subsamples of dietary fat were taken from the top, middle, and bottom, as well as, the center and peripheral of the container of fat. These samples were then homogenized and stored at -20°C to provide a representative sample for later analysis. Prior to the initiation of the experiment, pigs were fed a common post-weaning nursery diet.

Data and Sample Collection

Pigs were individually weighed on d 0, 7, 10, 22, 46, 53, and 56. Fecal grab samples were collected fresh from 0800 to 1000 h and 1600 to 1800 h on d 7 to 10 and d 53 to 56. Fecal samples were immediately stored at -20°C for later analysis.

Analytical Methods

Feed and fecal samples were homogenized, dried, and then finely ground through a 1 mm screen in a Retsch grinder (model ZMI; Retsch Inc., Newtown, PA). All feed analyses were performed in duplicate unless otherwise noted and repeated when the intraduplicate CV was greater than 1%. Acid hydrolyzed ether extract (method 2003.06; AOAC, 2007) was analyzed using a SoxCap SC 247 hydrolyzer and a Soxtec 255 semiautomatic extractor (FOSS North

America, Eden Prairie, MN). Dry matter was determined by drying samples in an oven at 105°C to a constant weight. Gross energy was determined using an isoperibolic bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). Benzoic acid (6.318 Mcal/kg; Parr Instrument Co.) was used as the standard for calibration and determined to contain 6.319 ± 0.005 Mcal of GE/kg. Titanium dioxide was determined by spectrophotometer (synergy 4; BioTek Instruments Inc., Winooski, VT) according to the method of Leone (1973). Dietary fat sources were analyzed in duplicate by a commercial laboratory (Barrow-Agee Laboratories, Memphis, TN) to determine fatty acid content (method Ce 1-62; AOCS, 2009), FFA (Ca 5a-40; AOCS, 2009), moisture and volatile matter (**MOVM;** Ca 2c-25; AOCS, 2009), insoluble impurities (**INIM**; Ca 3a-46, AOCS, 2009), unsaponifiable matter (**UNS**; Cb-53, AOCS, 2009), and initial peroxide value (**PV**; Cd 8b-90; AOCS, 2009).

Calculations

Basal diet DE was determined using the following equation: $DE_{basal\ diet} = \{DE_{CNTR\ diet} - [DE_{corn\ starch}$ (4.000 Mcal/kg; NRC, 1998) × proportion of corn starch added to the basal diet (5%)]} × 1.05. Energy value for each dietary fat source was determined according to the following equations: $DE_{dietary\ fat}$ (Mcal/kg) = {$DE_{test\ diet} - [DE_{basal\ diet}$ × (1 – proportion of dietary fat in the diet; 5%)]}/proportion of dietary fat in the diet; 5% (Villamide, 1996); $ME_{dietary\ fat}$ (Mcal/kg) = $DE_{deietary\ fat}$ × 98% (van Milgen et al., 2001); $NE_{dietary\ fat}$ (Mcal/kg) = $ME_{dietary\ fat}$ × 88% (van Milgen et al., 2001). All energy content values are reported on an as-fed basis. Iodine value was calculated from the fatty acid profile using the following equation: IV = [C16:1] × (0.95) + [C18:1] × (0.86) + [C18:2] × (1.732) + [C18:3] × (2.616) + [C20:1] × (0.795) + [C20:2]

× (1.57) + [C20:3] × (2.38) + [C20:4] × (3.19) + [C20:5] × (4.01) + [C22:4] × (2.93) + [C22:5] × (3.68) + [C22:6] × (4.64); brackets indicate percentage concentration (Meadus et al., 2010).

Statistical Analysis

These data were analyzed using PROC MIXED (SAS 9.4; SAS Inst. Inc., Cary, NC) with dietary treatment (n = 15) as a fixed effect, replicate (n = 2; 60 barrows each) as a random effect, and pig (n = 120) as the experimental unit. The comparison of the relationship between DE, ME, or NE content and the chemical composition of the 14 dietary fat sources were analyzed using PROC CORR and PROC REG. Correlation coefficients are reported as Pearson coefficients. Multivariate regression models were determined via stepwise selection with a significance stay level of 0.15. The dietary fat source multivariate factors included: fatty acid concentrations, SFA, MUFA, PUFA, Omega-3, Omega-6, IV, U:S, FFA, MOVA, INIM, UNS, MIU, and PV. The equation generated from each step of the regression analysis was reported sequentially. For each variable, normal distribution of residuals was tested using PROC UNIVARIATE.

To compare the observed dietary fat energy values herein to the previous equation reported by Powles et al. (1995), the standard error of prediction (prediction error [**PE**]) and prediction bias (**PBias**) were calculated using the following equations: PE = $\sqrt{}$ [(1/number of dietary fat treatments) × Σ (absolute differences between predicted and observed energy values)^2] and PBias = [(1/number of dietary fat treatments) × Σ (difference between predicted and observed energy values)] (smaller absolute value indicates greater accuracy of the equation; negative value indicates underestimation and positive value indicates overestimation; Lane et al., 2014).

Non-detectable fatty acid concentrations were treated in all statistical analyses as 0. All *P*-values < 0.050 were considered significant and *P*-values between 0.050 and 0.100 were considered trends.

Results

Determination of DE, ME and NE content of dietary fat sources

Dietary DE (Table 5.7) at 13 kg BW (d 7 to 10) was greater when dietary fat was added regardless of source in comparison to barrows fed CNTR ($P < 0.001$). The least dietary DE and estimated dietary fat DE, ME and NE were observed in pigs fed CORA-based diet (a moderately unsaturated but high FFA source) and the second least dietary DE and estimated dietary fat DE, ME and NE content were observed in pigs fed the COCO-based diet (the most saturated dietary fat source; $P < 0.001$). Across all the dietary fat sources tested at 13 kg BW, the average determined dietary fat DE was 8.42 Mcal/kg, ME was 8.26 Mcal/kg, and NE was 7.27 Mcal/kg; range in DE among the 14 dietary fat sources was 2.14 Mcal/kg (as-fed basis).

Adding dietary fat regardless of source increased the dietary DE (Table 5.8) at 50 kg BW (d 53 to 56) in comparison to pigs fed CNTR ($P < 0.001$). Dietary DE and estimated dietary fat DE, ME and NE were the greatest in the highly unsaturated dietary fat sources CANO and FLAX and the lowest DE, ME and NE were observed in AV- and CORA- (two sources with ≥ 7% FFA) based diets ($P < 0.001$). Across the 14 dietary fat sources tested at 50 kg BW, the average determined DE was 8.45 Mcal/kg, ME was 8.28, and NE was 7.29 Mcal/kg; the range in DE among the 14 dietary fat sources was 2.09 Mcal/kg (as-fed basis).

Relationship between dietary fat DE and chemical composition of dietary fat sources

At 13 kg BW, the dietary fat source DE content tended to be negatively correlated with Omega-6:Omega-3, FFA, and MOVM content ($P \leq 0.090$; Table 5.9). At 50 kg BW, the dietary fat source DE content was positively correlated with U:S ($P = 0.042$; Table 5.9). In addition, dietary fat DE tended to be positively correlated with linolenic acid and MUFA:SFA (**C18:3**; $P \leq 0.080$; Table 5.9).

The DE, ME and NE variation among dietary fat sources at 13 kg BW was largely explained ($R^2 = 0.82$) by a stepwise regression model with intercepts of 9.36, 9.18, and 8.08 Mcal/kg for DE, ME and NE respectively (Table 5.10). The model suggest that the energy value of dietary fat declines with increased FFA, Omega-6:Omega-3, INIM, and C20:0 content and increases with increasing C16:0 concentration ($P = 0.008$).

The variation in DE, ME and NE in 50 kg pigs was largely explained ($R^2 = 0.81$) by a stepwise regression model with intercepts of 8.35, 8.19, and 7.21 Mcal/kg for DE, ME and NE, respectively; Table 5.10). The model further suggested that the energy value of dietary fat was increased by increased dietary fat U:S and PUFA content and declined with increased FFA level and behenic acid (**C22:0**) concentration ($P = 0.002$).

Discussion

Impact of U:S on the DE content of dietary fat

Assigning accurate energy values to dietary fat sources not only allows pork producers to appropriately value dietary fat relative to other sources of energy, but also supports differentiation of available fat sources. Previous prediction equations used dietary fat U:S and FFA level as prediction variables (Powles et al., 1995; Rosero et al., 2015). In those equations, dietary fat DE content increased with increased U:S (Powles et al., 1995; NRC, 2012).

Unsaturated fatty acids are more soluble when exposed to bile salts, which may increase their incorporation into mixed micelles and facilitate subsequent absorption (Stahly, 1984; Wiseman et al., 1986). In the data reported herein, increased U:S resulted in increased dietary fat DE content at 50 kg BW, but not at 13 kg BW. The difference between the two stages of growth may possibly be due to bile secretion. Increased bile secretion was first purposed by Lloyd et al. (1957) to be the reason that fat digestion increased with pig age. Walker (1959) reported that the bile volume in the gall bladder is minimal in the young pig and is slow to increase over the early stages of growth. A gradual increase of bile salt secretion due to increased age in growing pigs was also reported by Harada et al. (1987). Thus, if bile salt exposure to fatty acids in the small intestine is greater with increased age, then the solubility of unsaturated fatty acids would similarly increase with age. However, the data reported in Powles et al. (1995) does not support this explanation, as they reported that the impact of U:S was greater in 12 kg pigs than in 30 to 90 kg pigs.

Impact of FFA on the DE content of dietary fat

The 14 fat sources evaluated in this experiment provided a wide range of U:S. They did not, however, vary much in FFA levels (≤ 13.4%). Despite this, FFA level was still a significant variable that decreased the energy value of dietary fat sources. For the younger pig, the negative effects of FFA were reduced if the dietary fat source was also highly unsaturated. Powles et al. (1995), using growing pigs, and Rosero et al. (2015) using lactating sows, also reported that saturated FFA lowered DE more than unsaturated FFA. Wiseman (1991) suggested that FFA, compared with esterified fatty acids could suppress bile salt secretion, resulting in a subsequent decrease of fatty acid incorporation into mixed micelles and thus absorption. Unsaturated FFA

are more effectively digested than their saturated FFA counterparts due to their being less hydrophobic (Liu et al., 2015) which in turn makes them less reliant on bile salts for emulsification and micelle incorporation (Liu et al., 2015).

The data reported herein agree with Powles et al. (1995) who also concluded that the negative effects of increased FFA is more pronounced in younger than older pigs. However, the magnitude of the impact was greater than that reported by Powles et al. (1995). They suggested that a 10% increase in FFA would reduce the predicted DE by 0.05 Mcal/kg; the data reported herein suggested that the impact was 0.97 Mcal/kg (at 13 kg BW) and 1.95 Mcal/kg (at 50 kg BW). The difference may be due to Powles et al. (1995) testing sources with a greater range of FFA level.

Estimation of the DE, ME and NE content of dietary fat

The NRC (2012) estimate of DE content is based on Powles et al. (1995). This series of experiments (Wiseman et al., 1990; Powles et al., 1993, 1994) used blends of dietary fat sources that ranged from 0.66 to 15.67 U:S and 0.8 to 81.8% FFA level. However, these experiments included dietary fat sources with primarily 16 or 18 carbon fatty acids. Therefore, the accuracy of the Powles et al. (1995) equation is unknown for shorter fatty acid sources (i.e. COCO) or longer fatty acid sources (i.e. FISH; NRC, 2012). Powles et al. (1995) related the DE content to chemical composition as follows: DE, kcal/kg = {36.898 – [(0.005 × FFA, g/kg) – (7.330 × $\exp^{(-0.906 \times U:S)}$]} / 4.184. Input of the analyzed composition of the 14 dietary fat sources in the Powles et al. (1995) equation generated an average predicted DE of 8.43 Mcal/kg (Table 5.11 and 5.12). The average observed DE content of the 14 dietary fat sources herein was 8.42 Mcal/kg at 13 kg BW and 8.45 Mcal/kg at 50 kg BW, respectively. Thus, the PBais of Powles et al. (1995)

equation to the observed DE content of dietary fat was minimal. However, at both 13 kg and 50 kg BW the Powles et al. (1995) equation underestimated the saturated fat sources COCO and PALM DE content and overestimated the CORA DE content to a large degree. Comparison of the equations generated herein to the Powles et al. (1995) equation is unfair, as these equations were fitted to the same dataset they are being compared to. Thus, validation of these equations in additional experiments is needed to determine if they are more precise than the Powles et al. (1995) equation across the wide range of dietary fat sources used by swine industry.

The approach herein for estimating dietary fat ME and NE content was modeled after the approach used by NRC (2012). Calculations of ME and NE from DE were based on diets containing 7% vegetable oil using indirect calorimetry (van Milgen et al., 2001). They estimated the conversion of DE to ME to be 98% and ME to NE to be 88%. The ME and NE estimates reported herein assume that the conversion of DE to NE is the same across all fat sources. The NRC (2012) ME and NE estimates are, of course, subject to the same errors.

A calibration of the NRC (2012) NE estimate of dietary fat was recently completed using a commercial scale growth-assay as reported by Boyd et al. (2015). Employing a diluent (bentonite, fine washed sand), Boyd et al. (2015) determined that the NE for choice white grease was 8.06 Mcal/kg at 38 to 67 kg BW and 8.50 Mcal/kg at 79 to 107 kg BW. These estimates are 10% and 14%, respectively, greater than those reported by the NRC (2012). The Boyd et al. (2015) calibration concluded that the energetic efficiency from DE to NE is greater than currently thought. Clearly, more work is needed on refining the estimation and prediction of dietary fat energy content in both the DE and NE systems.

Conclusion

The chemical composition of dietary fat explained a large degree of the variation observed in the energy content of dietary fat sources. However, the relationship between the energy content of dietary fat and the chemical composition of dietary fat was not the same at 13 kg and 50 kg BW, respectively. The Powles et al. (1995) equation accurately predicted the average DE content of the 14 sources. However, these data have identified 2 potential weaknesses of the equation. The Powles et al. (1995) equation incorrectly predicted the DE content of saturated sources of dietary fat that are composed of fatty acid chain lengths < 16 carbons and underestimated the negative impact of FFA. Further research is needed to validate the equations generated herein when in predicting the dietary fat DE among sources.

Literature cited

AOAC Int. 2007. Official methods of analysis. 18th rev. ed. AOAC Int., Gaithersburg, MD.

American Oil Chemists' Society (AOCS). 2009. Recommended practice Ca 3a-46, Ca 5a-40, Ca 2c-25, Cb-53, Ce 1-62, Cd 8b-90. In Official Methods and Recommended Practices of the AOCS. 6th ed. AOCS, Urbana, IL.

Boyd, R. D., C. E. Zier-Rush, M. McGrath, R. Palan, J. Picou, and E. van Heugten. 2015. Calibration of net energy for fat by growth assay in early and late phases of growth in pigs. J. Anim. Sci. 93(Suppl 2):73.

Galloway, S. T., and R. C. Ewan. 1989. Energy evaluation of tallow and oat groats for young swine. J. Anim. Sci. 67:1744-1750. doi:10.2527/jas1989.6771744x.

Harada, E., H. Kiriyama, E. Kobayashi, and H. Tsuchita. 1988. Postnatal development of biliary and pancreatic exocrine secretion in piglets. Comp. Biochem. Physiol. 91:43-51.

Jorgensen, H., S. K. Jensen, and B. O. Eggum. 1996. The influence of rapeseed oil on digestibility, energy metabolism and tissue fatty acid composition in pigs. Acta. Agri. Scand. A. Anim. Sci. 46:65-75.

Kil, D. Y., F. Ji, L. L. Stewart, R. B. Hinson, A. D. Beaulieu, G. L. Allee, J. F. Patience, J. E. Pettigrew, and H. H. Stein. 2011. Net energy of soybean oil and choice white grease in diets fed to growing and finishing pigs. J. Anim. Sci. 89:448-459. doi:10.2527/jas.2010-3233.

Knap, P. W. 2009. Allocation of resources to maintenance. In: W. M. Rauw, editor, Resource allocation theory applied to farm animal production. CAB Int., Cambridge, MA. p. 110-129.

Lane, D. M., D. Scott, M. Hebl, R. Guerra, D. Osherson, and H. Zimmer. 2014. Introduction to statistics. Rice Univ., Houston, TX. P. 474-476.

Liu, M. S., P. Green, J. J. Mann, S. I. Rapoport, and M. E. Sublette. 2015. Pathways of polyunsaturated fatty acid utilization: implications for brain function in neuropsychiatric health and disease. Brain. Res. 9:220-246. doi:10.1016/j.brainres.2014.11.059.

Lloyd, L. E., E. W. Crampton, and V. G. MacKay. 1957. The digestibility of ration nutrients by three vs. seven week old pigs. J. Anim. Sci. 16:383-388. doi:10.2527/jas1957.162383x.

Meadus, W. J., P. Duff, B. Uttaro, J. L. Aalhus, D. C. Rolland, L. L. Gibson, and M. E. Dugan. 2010. Production of docosahexaenoic acid (DHA) enriched bacon. J. Agric. Food Chem. 58:465-472. doi:10.1021/jf9028078.

Noblet, J. and X. S. Shi. 1993. Comparative digestibility of energy and nutrients in growing pigs fed ad libitum and adult sows fed at maintenance. Livest. Prod. Sci. 34:137-152. doi:10.1016/0301-6226(93)90042-G.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 1998. Nutrient requirements of swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

Patience, J. F. 2012. The influence of dietary energy on feed efficiency in grow-finish swine. In: J. F. Patience, editor, Feed Efficiency in Swine. Wageningen Academic Publishers, Wageningen, the Netherlands. p. 101-129.

Powles, J., J. Wiseman, D. J. A. Cole, and S. Jagger. 1995. Prediction of the apparent digestible energy value of fats given to pigs. Anim. Sci. 61:149-154. doi:1357-7298/95/45810149S20.00111.

Powles, J., J. Wiseman, D. J. A. Cole, and B. Hardy. 1994. Effect of chemical structure of fats upon their apparent digestible energy value when given to growing/finishing pigs. Anim. Prod. 58:411-417.

Powles, J., J. Wiseman, D. J. A. Cole, and B. Hardy. 1993. Effect of chemical structure of fats upon their apparent digestible energy value when given to growing/finishing pigs. Anim. Sci. 57:137-146. doi:10.1017/S000335610000670X.

Rosero, D. S., J. Odle, C. Arellano, R. D. Boyd, and E. van Heugten. 2015. Development of prediction equations to estimate the apparent digestible energy content of lipids when fed to lactating sows. J. Anim. Sci. 93:1165-1176. doi:10.2527/jas2014-8402.

Sauvant, D., J. M. Perex, and G. Tran. 2004. In: Tables of composition and nutritional value of feed materials, INRA, Paris, France, ed. Wageningen, the Netherlands: Wageningen Academic.

Stahly, T. 1984. Use of fats for growing pigs. In: J. Wiseman, editor, Fats in animal nutrition. Buttersworths, Boston, MA. P 313-331.

van Milgen, J., J. Noblet, and S. Dubois. 2001. Energetic efficiency of starch, protein, and lipid utilization in growing pigs. J. Nutr. 131:1309-1318.

Villamide, M. J. 1996. Methods of energy evaluation of feed ingredients for rabbits and their accuracy. Anim. Feed Sci. Tech. 57:211-223.

Walker, D. M. 1959. The development of the digestive system of the young animal. II. Carbohydrase enzyme development in the young pig. J. Agr. Sci. 52:357-363.

Wiseman, J., F. Salvador, and J. Craigon. 1991. The influence of free fatty acid content and degree of saturation on the apparent metabolizable energy value of fat fed to broilers. Poult. Sci. 70:1527-1533.

Wiseman, J., D. J. A. Cole, and B. Hardy. 1990. The dietary energy values of soya-bean oil, tallow, and their blends for growing/finishing pigs. Anim. Prod. 50:513-518.

Wiseman, J., D. J. A. Cole, F. G. Perry, B. G. Vernon, and B. C. Cooke. 1986. Apparent metabolizable energy values of fats for broiler chicks. Br. Poult. Sci. 27:561-576. doi:10.1080/0071668608416915.

Table 5.1. Ingredient and nutrient composition (as-fed basis) of experimental diets d 0 to 10

	Dietary treatments[1]														
Item	CNTR	AV	CANO	CWGA	CWGB	COCO	CORA	CORB	FISH	FLAX	PALM	POUF	SOYA	SOYB	TAL
Ingredient, %															
Corn	59.90	59.90	59.90	59.90	59.90	59.90	59.90	59.90	59.90	59.90	59.90	59.90	59.90	59.90	59.90
Soybean meal (46.5% CP)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Corn Starch	5.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Experimental dietary fat	-	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Whey, permeate	6.20	6.20	6.20	6.20	6.20	6.20	6.20	6.20	6.20	6.20	6.20	6.20	6.20	6.20	6.20
Plasma (spray-dried)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09
Monocalcium phosphate (21%)	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-lysine HCL	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43
DL-methionine	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
L-threonine	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
L-isoleucine	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
L-valine	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Trace mineral premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Santoquin[4]	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Analyzed composition															
DM, %	88.12	88.69	88.29	88.76	89.00	88.74	88.60	88.94	88.95	88.85	88.91	88.54	89.52	88.79	88.52
GE, Mcal/kg	3.94	4.21	4.15	4.12	4.17	4.13	4.10	4.18	4.15	4.17	4.20	4.17	4.18	4.21	4.17
Acid hydrolyzed ether extract, %	2.63	8.79	8.62	7.69	8.20	8.01	7.73	8.00	8.46	8.30	8.28	8.18	8.22	8.69	8.47

[1]CNTR = control, AV = animal-vegetable blend (sourced via Darling Pro Ingredients [Wahoo, NE]), CANO = canola oil (sourced via Bulk Apothecary [Aurora, OH]), CWGA = choice white grease source A (sourced via JBS [Marshalltown, IA]), CWGB = choice white grease source B (sourced via JBS [Worthington, MN]), COCO = coconut oil (sourced via Bulk Apothecary), CORA = corn oil source A (sourced via Feed Energy Co. [Des Moines, IA]), CORB = corn oil source B (sourced via Double S Liquid Feed Services [Danville, IL]), FISH = fish oil (sourced via Double S Liquid Feed Services), FLAX = flaxseed oil (sourced via Double S Liquid Feed

Services), PALM = palm oil (sourced via Bulk Apothecary), POUF = poultry fat (sourced via Boyer Valley Co. [Denison, IA]), SOYA = soybean oil source A (sourced via Status Foods [Memphis, TN]), SOYB = soybean oil source B (sourced via Bulk Apothecary), TAL = tallow (sourced via Darling Pro Ingredients [Omaha, NE]).

[2]Provided 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kilogram of diet.

[3]Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.023 mg vitamin B12 per kilogram of diet.

[4]Santoquin Mixture 6 (feed and forage antioxidant; Novus International, St. Charles, MO).

Table 5.2. Ingredient and nutrient composition (as-fed basis) of experimental diets d 46 to 56

	Dietary treatment[1]														
Item	CNTR	AV	CANO	CWGA	CWGB	COCO	CORA	CORB	FISH	FLAX	PALM	POUF	SOYA	SOYB	TAL
Ingredient, %															
Corn	68.41	68.41	68.41	68.41	68.41	68.41	68.41	68.41	68.41	68.41	68.41	68.41	68.41	68.41	68.41
Soybean meal (46.5% CP)	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50	22.50
Corn Starch	5.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Experimental dietary fat	-	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Monocalcium phosphate (21%)	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine HCL	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
DL-methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-threonine	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Trace mineral premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Santoquin[4]	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Analyzed composition															
DM, %	86.66	87.35	87.43	87.68	87.64	87.77	86.79	87.41	87.61	87.83	87.36	88.02	87.07	87.27	87.45
GE, Mcal/kg	3.92	4.07	4.11	4.13	4.09	4.06	4.05	4.10	4.06	4.13	4.10	4.08	4.11	4.09	4.09
Acid hydrolyzed ether extract, %	2.97	9.32	9.56	9.32	9.07	8.94	8.55	8.92	9.14	9.51	9.02	9.20	9.59	9.56	9.21

[1]CNTR = control, AV = animal-vegetable blend (sourced via Darling Pro Ingredients [Wahoo, NE]), CANO = canola oil (sourced via Bulk Apothecary [Aurora, OH]), CWGA = choice white grease source A (sourced via JBS [Marshalltown, IA]), CWGB = choice white grease source B (sourced via JBS [Worthington, MN]), COCO = coconut oil (sourced via Bulk Apothecary), CORA = corn oil source A (sourced via Feed Energy Co. [Des Moines, IA]), CORB = corn oil source B (sourced via Double S Liquid Feed Services [Danville, IL]), FISH = fish oil (sourced via Double S Liquid Feed Services), FLAX = flaxseed oil (sourced via Double S Liquid Feed Services), PALM = palm oil (sourced via Bulk Apothecary), POUF = poultry fat (sourced via Boyer Valley Co. [Denison, IA]),

SOYA = soybean oil source A (sourced via Status Foods [Memphis, TN]), SOYB = soybean oil source B (sourced via Bulk Apothecary), TAL = tallow (sourced via Darling Pro Ingredients [Omaha, NE]).

[2]Provided 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kilogram of diet.

[3]Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.023 mg vitamin B12 per kilogram of diet.

[4]Santoquin Mixture 6 (feed and forage antioxidant; Novus International, St. Charles, MO).

Table 5.3. Ingredient and nutrient composition (as-fed basis) of experimental diets d 10 to 46[1]

Item	Common diet
Ingredient, %	
Corn	62.34
Soybean meal (46.5% CP)	31.20
Soybean oil	2.50
Limestone	0.98
Monocalcium phosphate (21%)	1.25
Salt	0.60
L-lysine HCL	0.37
DL-methionine	0.16
L-threonine	0.15
Trace mineral premix[2]	0.20
Vitamin premix[3]	0.20
Santoquin[4]	0.06
Analyzed composition	
DM, %	87.14
GE, Mcal/kg	4.02
Acid hydrolyzed ether extract, %	5.60

[1]Feed to all pigs from d 10 to 46 regardless of experiment or treatment assigned.

[2]Provided 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kilogram of diet.

[3]Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.023 mg vitamin B12 per kilogram of diet.

[4]Santoquin Mixture 6 (feed and forage antioxidant; Novus International, St. Charles, MO).

Table 5.4. Analyzed chemical composition[1] of dietary fat sources[2]

Item	AV	CANO	CWGA	CWGB	COCO	CORA	CORB	FISH	FLAX	PALM	POUF	SOYA	SOYB	TAL
Free fatty acid, %	7.00	0.03	2.00	2.00	0.08	12.80	0.28	2.80	13.40	0.08	9.20	0.02	0.02	3.60
Moisture and volatile matter, %	0.06	0.02	0.16	0.12	0.02	0.42	0.02	0.34	0.30	0.02	0.32	0.02	0.02	0.06
Insoluble impurities, %	0.02	0.02	0.04	0.02	0.02	0.02	0.14	0.06	0.02	0.02	0.02	0.02	0.02	0.06
Unsaponifiable matter, %	0.41	0.67	0.51	0.39	0.23	0.47	0.39	0.69	0.76	0.17	0.82	0.43	0.35	0.31
MIU,[3] %	0.49	0.71	0.71	0.53	0.27	0.91	0.55	1.09	1.08	0.21	1.16	0.47	0.39	0.43
Initial peroxide value, mEq/kg	0.30	0.80	7.10	9.90	0.20	0.60	0.20	13.80	4.20	1.20	1.00	2.00	0.40	1.30

[1]Analysis via Barrow Agee Laboratories (Memphis, TN).

[2]AV = animal-vegetable blend (sourced via Darling Pro Ingredients [Wahoo, NE]), CANO = canola oil (sourced via Bulk Apothecary [Aurora, OH]), CWGA = choice white grease source A (sourced via JBS [Marshalltown, IA]), CWGB = choice white grease source B (sourced via JBS [Worthington, MN]), COCO = coconut oil (sourced via Bulk Apothecary), CORA = corn oil source A (sourced via Feed Energy Co. [Des Moines, IA]), CORB = corn oil source B (sourced via Double S Liquid Feed Services [Danville, IL]), FISH = fish oil (sourced via Double S Liquid Feed Services), FLAX = flaxseed oil (sourced via Double S Liquid Feed Services), PALM = palm oil (sourced via Bulk Apothecary), POUF = poultry fat (sourced via Boyer Valley Co. [Denison, IA]), SOYA = soybean oil source A (sourced via Status Foods [Memphis, TN]), SOYB = soybean oil source B (sourced via Bulk Apothecary), TAL = tallow (sourced via Darling Pro Ingredients [Omaha, NE]).

[3]MIU = moisture, impurities, and unsaponifiables.

Table 5.5. Analyzed fatty acid concentrations (%)[1] of dietary fat sources[2]

Item	AV	CANO	CWGA	CWGB	COCO	CORA	CORB	FISH	FLAX	PALM	POUF	SOYA	SOYB	TAL
C5:0	ND[4]	ND	ND	ND	0.46	ND	ND	ND	ND	ND	ND	ND	ND	ND
C8:0	ND	ND	ND	ND	6.17	ND	ND	ND	ND	ND	ND	ND	ND	ND
C10:0	ND	ND	ND	ND	5.39	ND	ND	ND	ND	ND	ND	ND	ND	ND
C12:0	ND	ND	ND	ND	48.46	ND	ND	0.11	ND	0.19	ND	ND	ND	ND
C14:0	1.63	ND	1.31	1.33	19.75	ND	0.07	9.88	ND	1.03	0.74	0.07	0.07	2.78
C14:1	0.21	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.14	ND	ND	0.54
C15:0	0.14	ND	ND	ND	ND	ND	ND	0.73	ND	ND	ND	ND	ND	0.43
C16:0	22.39	4.16	22.47	22.35	9.44	11.92	10.60	20.33	5.20	44.19	18.89	10.79	10.55	24.08
C16:1	2.92	0.20	2.49	2.52	ND	0.09	0.08	11.66	ND	0.15	3.99	0.08	0.08	2.48
C16:2	ND	ND	ND	ND	ND	ND	ND	1.43	ND	ND	ND	ND	ND	ND
C17:0	0.46	ND	0.33	0.33	ND	ND	0.11	0.82	ND	0.10	0.24	0.10	0.10	1.22
C17:1	0.41	0.15	ND	ND	ND	ND	ND	0.25	ND	ND	ND	ND	ND	ND
C18:0	10.45	1.80	11.21	10.97	9.08	1.71	4.30	3.49	3.20	4.47	6.31	3.78	3.78	20.29
C18:1	45.25	63.36	42.15	42.34	1.07	27.20	22.94	9.28	17.00	39.42	34.53	22.00	23.50	41.59
C18:2	13.41	19.28	16.54	16.72	0.06	56.84	53.37	1.15	14.90	9.52	31.78	54.19	52.27	2.81
C18:3	0.62	8.41	0.60	0.60	ND	1.35	7.61	1.34	59.60	0.19	2.06	7.84	8.14	0.31
C18:4	ND	ND	ND	ND	ND	ND	ND	2.01	ND	ND	ND	ND	ND	ND
C19:0	ND	ND	ND	ND	ND	ND	0.11	ND	0.20	ND	ND	ND	ND	ND
C19:1	ND	0.36	ND	ND	ND	ND	ND	0.42	ND	ND	ND	ND	ND	0.11
C20:0	0.15	0.58	0.16	0.16	0.12	0.36	0.31	0.24	ND	0.36	ND	0.28	0.27	0.12
C20:1	0.67	1.10	0.82	0.83	ND	0.26	0.18	0.86	ND	0.13	0.25	0.17	0.17	0.23
C20:2	0.57	ND	0.83	0.84	ND	ND	ND	0.20	ND	ND	0.23	ND	ND	ND
C20:3	ND	ND	0.13	0.13	ND	ND	ND	1.36	ND	ND	0.62	ND	ND	ND
C20:4	0.24	ND	0.36	0.36	ND	ND	ND	1.36	ND	ND	0.62	ND	ND	ND
C20:5	ND	ND	ND	ND	ND	ND	ND	14.32	ND	ND	ND	ND	ND	ND
C22:0	ND	0.31	ND	ND	ND	0.13	0.34	0.16	ND	ND	ND	0.33	0.32	ND
C22:1	ND	ND	ND	ND	ND	ND	ND	0.10	ND	ND	ND	ND	ND	ND
C22:3	ND	ND	ND	ND	ND	ND	ND	0.40	ND	ND	ND	ND	ND	ND
C22:4	ND	ND	0.17	0.16	ND	ND	ND	0.23	ND	ND	ND	ND	ND	ND
C22:5	ND	0.15	ND	ND	ND	0.16	ND	2.81	ND	ND	ND	ND	ND	ND
C22:6	ND	ND	ND	ND	ND	ND	ND	8.22	ND	ND	ND	ND	ND	ND
C24:1	ND	0.13	ND	ND	ND	ND	ND	0.25	ND	ND	ND	ND	ND	ND
Other	0.46	ND	0.36	0.35	ND	ND	ND	7.56	ND	0.23	0.23	0.13	0.14	3.01

[1]Analysis via Barrow Agee Laboratories (Memphis, TN).

[2]AV = animal-vegetable blend (sourced via Darling Pro Ingredients [Wahoo, NE]), CANO = canola oil (sourced via Bulk Apothecary [Aurora, OH]), CWGA = choice white grease source A (sourced via JBS [Marshalltown, IA]), CWGB = choice white grease source B (sourced via JBS [Worthington, MN]), COCO = coconut oil (sourced via Bulk Apothecary), CORA = corn oil source

A (sourced via Feed Energy Co. [Des Moines, IA]), CORB = corn oil source B (sourced via Double S Liquid Feed Services [Danville, IL]), FISH = fish oil (sourced via Double S Liquid Feed Services), FLAX = flaxseed oil (sourced via Double S Liquid Feed Services), PALM = palm oil (sourced via Bulk Apothecary), POUF = poultry fat (sourced via Boyer Valley Co. [Denison, IA]), SOYA = soybean oil source A (sourced via Status Foods [Memphis, TN]), SOYB = soybean oil source B (sourced via Bulk Apothecary), TAL = tallow (sourced via Darling Pro Ingredients [Omaha, NE]).

[3]Valeric acid (C5:0), caproic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), hexadecadienoic acid (C16:2), margaric acid (C17:0), margaroleic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), octadecatetraenoic acid (C18:4), nonadecenoic acid (C19:1), arachidic acid (C20:0), gadoleic acid (C20:1), eicosadienoic acid (C20:2), homo-γ linolenic acid (20:3), arachidonic acid (C20:4), eicosapentaenoic acid (C20:5), behenic acid (C22:0), erucic acid (C22:1), docosatrienoic acid (C22:3), docosatetraenoic acid (C22:4), docosapentaenoic acid (C22:5), docosahexaenoic acid (C22:6), nervonic acid (C24:1).

[4]ND = non-detectable.

Table 5.6. Analyzed fatty acid composition and characteristics[1] of dietary fat sources[2]

Item	AV	CANO	CWGA	CWGB	COCO	CORA	CORB	FISH	FLAX	PALM	POUF	SOYA	SOYB	TAL
Omega-3, %	0.62	8.56	0.73	0.73	0.00	1.51	7.61	29.08	59.60	0.19	2.06	7.84	8.14	0.31
Omega-6, %	14.22	19.28	17.90	18.08	0.06	56.84	53.37	2.94	14.90	9.52	32.63	54.19	52.57	2.81
Omega-6:Omega-3	22.94	2.25	24.52	24.77	NC	37.64	7.10	0.10	0.25	50.11	15.84	6.91	6.46	9.06
MUFA, %	49.46	65.30	45.46	45.69	1.07	27.55	23.20	22.82	17.00	39.70	38.91	22.25	23.75	44.95
PUFA, %	14.84	27.84	18.63	18.81	0.06	58.35	60.98	33.85	74.40	9.71	34.69	62.03	60.71	3.12
SFA, %	35.22	6.85	35.36	35.14	98.87	14.12	15.84	35.76	8.60	50.34	26.18	15.59	15.39	48.92
MUFA:PUFA	3.33	2.35	2.44	2.43	17.83	0.47	0.38	0.67	0.23	4.09	1.12	0.36	0.39	14.41
MUFA:SFA	1.40	9.53	1.28	1.30	0.01	1.95	1.46	0.64	1.97	0.79	1.49	1.43	1.54	0.92
PUFA:SFA	0.42	4.06	0.52	0.54	0.00	4.13	3.85	0.95	8.66	0.19	1.33	3.98	3.94	0.06
IV[3], g/ 100 g	68.7	111.5	72.7	73.2	1.0	126.3	132.3	137.4	196.2	51.1	96.5	133.5	132.8	44.0
U:S[4]	1.83	13.60	1.80	1.84	0.01	6.08	5.31	1.58	10.63	0.98	2.81	5.41	5.49	0.98

[1]Analysis via Barrow Agee Laboratories (Memphis, TN).

[2]AV = animal-vegetable blend (sourced via Darling Pro Ingredients [Wahoo, NE]), CANO = canola oil (sourced via Bulk Apothecary [Aurora, OH]), CWGA = choice white grease source A (sourced via JBS [Marshalltown, IA]), CWGB = choice white grease source B (sourced via JBS [Worthington, MN]), COCO = coconut oil (sourced via Bulk Apothecary), CORA = corn oil source A (sourced via Feed Energy Co. [Des Moines, IA]), CORB = corn oil source B (sourced via Double S Liquid Feed Services [Danville, IL]), FISH = fish oil (sourced via Double S Liquid Feed Services), FLAX = flaxseed oil (sourced via Double S Liquid Feed Services), PALM = palm oil (sourced via Bulk Apothecary), POUF = poultry fat (sourced via Boyer Valley Co. [Denison, IA]), SOYA = soybean oil source A (sourced via Status Foods [Memphis, TN]), SOYB = soybean oil source B (sourced via Bulk Apothecary), TAL = tallow (sourced via Darling Pro Ingredients [Omaha, NE]).

[3]Iodine value calculated from fatty acid composition: (IV) = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.795 + [C20:2] × 1.57 + [C20:3] × 2.38 + [C20:4] × 3.19 + [C20:5] × 4.01 + [C22:4] × 2.93 + [C22:5] × 3.68 + [C22:6] × 4.64; brackets indicate percentage concentration (Meadus et al., 2010).

[4]U:S = unsaturated fatty acid concentration to SFA concentration.

Table 5.7. Determination of DE, ME and NE content of dietary fat sources (Mcal/kg; as-fed basis) based on the apparent total tract digestion of GE at 13 kg BW[1]

	Dietary treatment[2]																
Item	CNTR	AV	CANO	CWGA	CWGB	COCO	CORA	CORB	FISH	FLAX	PALM	POUF	SOYA	SOYB	TAL	SEM	*P*-value
Diet (Mcal/kg)																	
GE	3.94	4.21	4.15	4.12	4.17	4.13	4.10	4.18	4.15	4.17	4.20	4.17	4.18	4.21	4.17	-	-
DE	3.70^{f}	3.94^{ab}	3.93^{abc}	3.91^{bcd}	3.93^{abc}	3.88^{d}	3.84^{e}	3.92^{abc}	3.93^{abc}	3.90^{cd}	3.94^{ab}	3.93^{abc}	3.95^{a}	3.95^{ab}	3.91^{bcd}	0.01	<0.001
Dietary fat (Mcal/kg)																	
DE[3]	-	8.81^{abc}	8.59^{abc}	8.32^{bcd}	8.67^{abc}	7.65^{d}	6.90^{e}	8.52^{abc}	8.69^{abc}	8.06^{cd}	8.81^{ab}	8.67^{abc}	9.04^{a}	8.99^{ab}	8.33^{bcd}	0.25	<0.001
ME[4]	-	8.63^{abc}	8.42^{abc}	8.15^{bcd}	8.49^{abc}	7.49^{d}	6.58^{e}	8.35^{abc}	8.52^{abc}	7.90^{cd}	8.63^{ab}	8.49^{abc}	8.86^{a}	8.81^{ab}	8.16^{bcd}	0.24	<0.001
NE[5]	-	7.59^{abc}	7.41^{abc}	7.17^{bcd}	7.47^{abc}	6.59^{d}	5.95^{e}	7.35^{abc}	7.50^{abc}	6.95^{cd}	7.60^{ab}	7.47^{abc}	7.80^{a}	7.76^{ab}	7.18^{bcd}	0.21	<0.001

[1]Determined via 120 pigs (8 pigs/treatment) with a d 7 BW of 12.3 ± 0.2 kg and a d 10 BW of 13.8 ± 0.4 kg.

[2]Each experimental diet included 95% of a corn-soybean meal basal diet and then 5% of either: corn starch (CNTR), animal-vegetable blend (AV), canola oil (CANO), choice white grease source A (CWGA), choice white grease source B (CWGB), coconut oil (COCO), corn oil source A (CORA), corn oil source B (CORB), fish oil (FISH), flaxseed oil (FLAX), palm oil (PALM), poultry fat (POUF), soybean oil source A (SOYA), soybean oil source B (SOYB), or tallow (TAL).

[3]$DE_{dietary\ fat}$ (Mcal/kg) = {$DE_{test\ diet}$ – [$DE_{basal\ diet}$ (3.68 Mcal/kg) × (1 – proportion of dietary fat in the diet; 5%)]}/proportion of dietary fat in the diet; 5% (Villamide, 1996).

[4]$ME_{dietary\ fat}$ (Mcal/kg) = DE × 98% (van Milgen et al., 2001; NRC, 2012).

[5]$NE_{dietary\ fat}$ (Mcal/kg) = ME × 88% (van Milgen et al., 2001; NRC, 2012).

Table 5.8. Determination of DE, ME and NE content of dietary fat sources (Mcal/kg; as-fed basis) based on the apparent total tract digestion of GE at 50 kg BW[1]

	Dietary treatment[2]															
Item	CNTR	AV	CANO	CWGA	CWGB	COCO	CORA	CORB	FISH	FLAX	PALM	POUF	SOYA	SOYB	TAL	SEM	*P*-value
Diet (Mcal/kg)																	
GE	3.89	4.07	4.11	4.13	4.09	4.06	4.05	4.10	4.06	4.13	4.10	4.08	4.11	4.09	4.09	-	-
DE	3.65^{i}	3.81^{h}	3.92^{a}	3.91^{ab}	3.88^{cd}	3.84^{fgh}	3.81^{h}	3.87^{cde}	3.83^{gh}	3.91^{ab}	3.86^{cdef}	3.85^{efg}	3.89^{abc}	3.85^{defg}	3.85^{defg}	0.02	<0.001
Dietary fat (Mcal/kg)																	
DE[3]	-	7.51^{g}	9.53^{a}	9.31^{a}	8.72^{bc}	7.97^{efg}	7.43^{g}	8.55^{bcd}	7.77^{fg}	9.43^{a}	8.50^{bcde}	8.14^{def}	9.05^{ab}	8.18^{cdef}	8.22^{cdef}	0.31	<0.001
ME[4]	-	7.36^{g}	9.34^{a}	9.12^{a}	8.55^{bc}	7.81^{efg}	7.28^{g}	8.38^{bcd}	7.61^{fg}	9.24^{a}	8.33^{bcde}	7.97^{def}	8.87^{ab}	8.02^{cdef}	8.05^{cdef}	0.31	<0.001
NE[5]	-	6.48^{g}	8.22^{a}	8.03^{a}	7.52^{bc}	6.87^{efg}	6.41^{g}	7.38^{bcd}	6.70^{fg}	8.13^{a}	7.33^{bcde}	7.02^{def}	7.80^{ab}	7.06^{cdef}	7.09^{cdef}	0.27	<0.001

[1]Determined via 120 pigs (8 pigs/treatment) with a d 53 BW of 49.1 ± 2.2 kg and a d 56 BW of 51.7 ± 1.7 kg.

[2]Each experimental diet included 95% of a corn-soybean meal basal diet and then 5% of either: corn starch (CNTR), animal-vegetable blend (AV), canola oil (CANO), choice white grease source A (CWGA), choice white grease source B (CWGB), coconut oil (COCO), corn oil source A (CORA), corn oil source B (CORB), fish oil (FISH), flaxseed oil (FLAX), palm oil (PALM), poultry fat (POUF), soybean oil source A (SOYA), soybean oil source B (SOYB), or tallow (TAL).

[3]$DE_{dietary\ fat}$ (Mcal/kg) = {$DE_{test\ diet}$ – [$DE_{basal\ diet}$ (3.62 Mcal/kg) × (1 – proportion of dietary fat in the diet; 5%)]}/proportion of dietary fat in the diet; 5% (Villamide, 1996).

[4]$ME_{dietary\ fat}$ (Mcal/kg) = DE × 98% (van Milgen et al., 2001; NRC, 2012).

[5]$NE_{dietary\ fat}$ (Mcal/kg) = ME × 88% (van Milgen et al., 2001; NRC, 2012).

Table 5.9. Correlation coefficients (*r*) between dietary fatty acid composition and estimated dietary fat DE content (Mcal/kg)

	Dietary fat DE (Mcal/kg)	
Item	13 kg[1]	50 kg[2]
Fatty acid[3], %		
Linoleic acid (C18:3)	NS[7]	0.489*
Omega-3, %	NS	NS
Omega-6, %	NS	NS
Omega-6:Omega-3	-0.468*	NS
MUFA, %	NS	NS
PUFA, %	NS	NS
SFA, %	NS	NS
MUFA:PUFA	NS	NS
MUFA:SFA	NS	0.483*
PUFA:SFA	NS	NS
IV, (Meadus, 2010)[4] g/ 100 g	NS	NS
U:S[5]	NS	0.549**
Free fatty acid, %	-0.530*	NS
Moisture and volatile matter, %	-0.498*	NS
Insoluble impurities, %	NS	NS
Unsaponifiable matter, %	NS	NS
MIU,[6] %	NS	NS
Initial peroxide value, mEq/kg	NS	NS

*Probability value of obtaining the observed coefficient ($P \leq 0.100 \geq 0.050$).

**Probability value of obtaining the observed coefficient ($P \leq 0.050$).

[1]Determined via 120 pigs (8 pigs/treatment) with a d 7 BW of 12.3 ± 0.2 kg and a d 10 BW of 13.8 ± 0.4 kg.

[2]Determined via 120 pigs (8 pigs/treatment) with a d 53 BW of 49.1 ± 2.2 kg and a d 56 BW of 51.7 ± 1.7 kg.

[3]Other than linoleic acid (C18:3, %), no other dietary fatty acid concentrations were correlated with the DE content of dietary fat ($P \geq 0.101$).

[4]Iodine value calculated from fatty acid composition: (IV) = [C16:1] × 0.95 + [C18:1] × 0.86 + [C18:2] × 1.732 + [C18:3] × 2.616 + [C20:1] × 0.795 + [C20:2] × 1.57 + [C20:3] × 2.38 + [C20:4] × 3.19 + [C20:5] × 4.01 + [C22:4] × 2.93 + [C22:5] × 3.68 + [C22:6] × 4.64; brackets indicate percentage concentration (Meadus et al., 2010).

[5]Unsaturated fatty acid concentration to SFA concentration.

[6]MIU = moisture, impurities, and unsaponifiables.

[7]NS = non-significant ($P > 0.100$).

Table 5.10. Relationship between dietary fat DE, ME and NE (Mcal/kg; as-fed basis) content and chemical composition[1] of dietary fat source as determined via stepwise regression analysis

Item	Equation	Mean square error	R^2	*P*-value
13 kg[2]				
DE	= 8.671 – [0.063 × (FFA)]	0.258	0.282	0.051
	= 8.967 – [0.073 × (FFA)] – [0.012 × Omega-6:Omega-3]	0.164	0.581	0.008
	= 9.353 – [0.092 × (FFA)] – [0.013 × Omega-6:Omega-3] – [1.290 × (C20:0)]	0.140	0.675	0.008
	= 9.656 – [0.104 × (FFA)] – [0.015 × Omega-6:Omega-3] – [1.389 × (C20:0)] – [5.294 × (INIM)]	0.118	0.755	0.008
	= 9.363 – [0.097 × (FFA)] – [0.016 × Omega-6:Omega-3] – [1.240 × (C20:0)] – [5.054 × (INIM)] + [0.014 × (C16:0)]	0.099	0.815	0.008
ME	= 8.498 – [0.062 × (FFA)]	0.248	0.282	0.051
	= 8.787 – [0.071 × (FFA)] – [0.012 × Omega-6:Omega-3]	0.157	0.581	0.008
	= 9.353 – [0.090 × (FFA)] – [0.013 × Omega-6:Omega-3] – [1.265 × (C20:0)]	0.135	0.675	0.008
	= 9.463 – [0.102 × (FFA)] – [0.015 × Omega-6:Omega-3] – [1.361 × (C20:0)] – [5.188 × (INIM)]	0.113	0.755	0.008
	= 9.176 – [0.095 × (FFA)] – [0.016 × Omega-6:Omega-3] – [1.215 × (C20:0)] – [4.953 × (INIM)] + [0.014 × (C16:0)]	0.096	0.815	0.008
NE	= 7.478 – [0.055 × (FFA)]	0.192	0.282	0.051
	= 7.732 – [0.063 × (FFA)] – [0.010 × Omega-6:Omega-3]	0.122	0.581	0.008
	= 8.066 – [0.079 × (FFA)] – [0.011 × Omega-6:Omega-3] – [1.113 × (C20:0)]	0.104	0.675	0.008
	= 8.327 – [0.089 × (FFA)] – [0.013 × Omega-6:Omega-3] – [1.198 × (C20:0)] – [4.566 × (INIM)]	0.087	0.755	0.008
	= 8.075 – [0.093 × (FFA)] – [0.014 × Omega-6:Omega-3] – [1.070 × (C20:0)] – [4.359 × (INIM)] + [0.013 × (C16:0)]	0.074	0.815	0.008
50 kg[3]				
DE	= 8.050 + [0.096 × U:S]	0.358	0.302	0.042
	= 8.190 + [0.110 × U:S] – [0.052 × (FFA)]	0.319	0.429	0.046
	= 8.439 + [0.189 × U:S] – [0.107 × (FFA)] – [3.232 × (C22:0)]	0.222	0.639	0.014
	= 8.357 + [0.189 × U:S] – [0.195 × (FFA)] – [6.768 × (C22:0)] + [0.024 × (PUFA)]	0.128	0.813	0.003
ME	= 7.889 + [0.094 × U:S]	0.344	0.302	0.042
	= 8.026 + [0.108 × U:S] – [0.052 × (FFA)]	0.307	0.429	0.046
	= 8.270 + [0.185 × U:S] – [0.105 × (FFA)] – [3.168 × (C22:0)]	0.217	0.639	0.014
	= 8.190 + [0.185 × U:S] – [0.191 × (FFA)] – [6.633 × (C22:0)] + [0.023 × (PUFA)]	0.123	0.813	0.003
NE	= 6.942 + [0.083 × U:S]	0.266	0.302	0.042
	= 7.063 + [0.095 × U:S] – [0.045 × (FFA)]	0.237	0.429	0.046
	= 7.277 + [0.163 × U:S] – [0.092 × (FFA)] – [2.787 × (C22:0)]	0.165	0.639	0.014
	= 7.207 + [0.163 × U:S] – [0.168 × (FFA)] – [5.836 × (C22:0)] + [0.021 × (PUFA)]	0.095	0.813	0.003

[1]C16:0 = palmitic acid (%); C20:0 =arachidic acid (%); C22:0 = behenic acid (%); FFA = free fatty acid (%); INIM = insoluble impurities (%); U:S = unsaturated to saturated fatty acid ratio; parenthesis indicate concentration (%).

[2]Determined via 120 pigs (8 pigs/treatment) with a d 7 BW of 12.3 ± 0.2 kg and a d 10 BW of 13.8 ± 0.4 kg.

[3]Determined via 120 pigs (8 pigs/treatment) with a d 53 BW of 49.1 ± 2.2 kg and a d 56 BW of 51.7 ± 1.7 kg.

Table 5.11. Comparison of predicted versus observed DE (Mcal/kg) values at 13 kg

Item	Observed DE[1]	Powles et al. (1995) predicted DE[2]	Δ DE[3]	Predicted DE[4]	Δ DE
Source					
Animal-vegetable blend	8.81	8.40	-0.41	8.34	-0.46
Canola oil	8.59	8.82	0.23	8.56	-0.02
Choice white grease source A	8.32	8.45	0.13	8.69	0.37
Choice white grease source B	8.67	8.46	-0.21	8.79	0.12
Coconut oil	7.65	7.08	-0.56	7.64	-0.01
Corn oil source A	6.90	8.66	1.76	7.14	0.24
Corn oil source B	8.52	8.80	0.28	8.28	-0.24
Fish oil	8.69	8.37	-0.32	8.78	0.08
Flax oil	8.06	8.66	0.60	8.03	-0.03
Palm oil	8.81	8.10	-0.71	8.62	-0.18
Poultry fat	8.67	8.57	-0.10	8.38	-0.28
Soybean oil source A	9.04	8.81	-0.23	8.95	-0.08
Soybean oil source B	8.99	8.81	-0.18	8.97	-0.02
Tallow	8.33	8.06	-0.27	8.76	0.43
Predication error[5]	-	1.60	-	0.68	-
Prediction bias[6]	-	0.01	-	-0.01	-

[1]Determined via 120 pigs (8 pigs/treatment) with a d 7 BW of 12.3 ± 0.2 kg and a d 10 BW of 13.8 ± 0.4 kg.

[2]DE (kcal/kg) = [36.898 – (0.005 × free fatty acid, g/kg) – 7.330 × $^{e-0.906 \times \text{unsaturated fatty acid to SFA ratio}}$)]/0.004184 (Powles et al., 1995); refer to table 5.5 and 5.6 for dietary fatty acid and chemical composition.

[3]Delta DE (Mcal/kg) = predicted DE (Mcal/kg) – observed DE (Mcal/kg).

[4]DE (Mcal/kg) = 9.363 – [0.097 × FFA, %] – [0.016 × Omega-6:Omega-3] – [1.240 × arachidic acid, %] – [5.054 × insoluble impurities, %] + [0.014 × palmitic acid, %]; refer to table 5.10.

[5]Prediction error = $\sqrt{}$ [(1/number of dietary fat treatments) × Σ (absolute differences between predicted and observed energy values)^2] (Lane et al., 2014).

[6]Prediciton bias = [(1/number of dietary fat treatments) × Σ (difference between predicted and observed energy values)] (smaller absolute value indicates greater accuracy of the equation; negative value indicates underestimation and positive value indicates overestimation; Lane et al., 2014).

Table 5.12. Comparison of predicted versus observed DE (Mcal/kg) values at 50 kg

Item	Observed DE[1]	Powles et al. (1995) predicted DE[2]	Δ DE[3]	Predicted DE[4]	Δ DE
Source					
Animal-vegetable blend	7.51	8.40	0.89	7.69	0.19
Canola oil	9.53	8.82	-0.71	9.52	-0.01
Choice white grease source A	9.31	8.45	-0.86	8.75	-0.56
Choice white grease source B	8.72	8.46	-0.26	8.77	0.04
Coconut oil	7.97	7.08	-0.89	8.34	0.38
Corn oil source A	7.43	8.66	1.23	7.54	0.11
Corn oil source B	8.55	8.80	0.25	8.50	-0.05
Fish oil	7.77	8.37	0.60	7.85	0.09
Flax oil	9.43	8.66	-0.77	9.54	0.11
Palm oil	8.50	8.10	-0.40	8.76	0.26
Poultry fat	8.14	8.57	0.43	7.93	-0.21
Soybean oil source A	9.05	8.81	-0.24	8.66	-0.39
Soybean oil source B	8.18	8.81	0.63	8.71	0.53
Tallow	8.22	8.06	-0.16	7.92	-0.30
Predication error[5]	-	2.22	-	0.86	-
Prediction bias[6]	-	-0.02	-	0.01	-

[1]Determined via 120 pigs (8 pigs/treatment) with a d 53 BW of 49.1 ± 2.2 kg and a d 56 BW of 51.7 ± 1.7 kg.

[2]DE (kcal/kg) = [36.898 – (0.005 × free fatty acid, g/kg) – 7.330 × $^{e-0.906 \times \text{unsaturated fatty acid to SFA ratio}}$)]/0.004184 (Powles et al., 1995); refer to table 5.5 and 5.6 for dietary fatty acid and chemical composition.

[3]Delta DE (Mcal/kg) = predicted DE (Mcal/kg) – observed DE (Mcal/kg).

[4]DE (Mcal/kg) = 8.357 + [0.189 × unsaturated fatty acid:SFA] – [0.195 × FFA, %] – [6.768 × behenic acid, %] + [0.024 × PUFA, %]; refer to table 5.10.

[5]Prediction error = $\sqrt{}$ [(1/number of dietary fat treatments) × Σ (absolute differences between predicted and observed energy values)^2] (Lane et al., 2014).

[6]Prediciton bias = [(1/number of dietary fat treatments) × Σ (difference between predicted and observed energy values)] (smaller absolute value indicates greater accuracy of the equation; negative value indicates underestimation and positive value indicates overestimation; Lane et al., 2014).

CHAPTER VI

IMPACT OF ENDOGENOUS LOSSES OF FAT ON THE ENERGY CONTENT OF DIETARY FAT IN 13 AND 50 KG PIGS

A paper in preparation for submission to the *Journal of Animal Science*

T. A. Kellner and J. F. Patience*

*Department of Animal Science, Iowa State University, Ames, 50011, USA

Abstract

The objectives of this experiment were to estimate basal ELF by feeding a fat-free diet; to determine the apparent total tract digestibility (ATTD) and STTD of 14 dietary fat sources; and to quantify the underestimation of the energy content of dietary fat due to not accounting for ELF. Determination of ATTD and STTD of AEE were done using 120 Genetriporc 6.0 × Genetiporc F25 (PIC, Inc., Hendersonville, TN) barrows (in 2 sequential cohorts of 60 barrows each). Barrows were randomly allotted to 1 of 15 dietary treatments. Each experimental diet included 95% of a corn-soybean meal basal diet plus 5% of either: corn starch (as a control) or 1 of 14 dietary fat sources. The 14 dietary fat sources (animal-vegetable blend, canola oil, choice white grease source A, choice white grease source B, coconut oil, corn oil source A, corn oil source B, fish oil, flaxseed oil, palm oil, poultry fat, soybean oil source A, soybean oil source B, and tallow) were selected to provide a diverse range of fatty acid composition. Pigs were limit fed experimental diets from d 0 to 10 and d 46 to 56 providing a 7 d adaption for fecal collection on d 7 to 10 (13 kg BW) and d 53 to 56 (50 kg BW). Estimated ELF at 9 kg BW was 4.17 g/kg of DM intake ($P < 0.001$). Estimated ELF at 38 kg BW was 6.67 g/kg of DM intake ($P = 0.002$).

Adding 5% dietary fat regardless of source compared to pigs fed 5% corn starch increased the ATTD and STTD of AEE at both fecal collection time points ($P < 0.001$). At 13 kg BW, the STTD of AEE was the greatest in barrows fed CANO-, CWGA-, and FISH-based diets and was the least in pigs fed PALM- and TAL-based diets ($P < 0.001$). The average STTD of AEE among the 14 dietary fat sources at 13 kg BW was 93.7% and the range was 3.20%. At 50 kg BW, ATTD and STTD of AEE was the greatest in pigs fed a CANO-based diet and the least in pigs fed a CORA-based diet ($P < 0.001$). The average of STTD of AEE among the 14 dietary fat sources at 50 kg BW was 96.8% and the range was 4.22%. On average ELF accounted for 43.1% and 68.0% of the fecal AEE both fecal collection points, respectively. The substantial proportion of AEE contained in feces that is of ELF origin and not of dietary origin implies that the current estimates of the DE content of dietary fat are underestimated. Not correcting for ELF, resulted in underestimating dietary fat DE content by 0.42 and 0.60 Mcal/kg at 13 and 50 kg of BW, respectively.

Introduction

Digestibility of fat is determined by the difference between the fat concentration in the diet and in the feces, or at the terminal ileum (Oresanya et al., 2007). However, not all of the fat contained in the feces is of dietary origin (Adams and Jensen, 1984; Gutierrez et al., 2016). Endogenous losses of fat (**ELF**) can result from sloughed intestinal cells, microbes, and digestive secretions (Kil et al., 2010). Endogenous losses of fat will contain both basal losses (not specific to a particular diet; used to estimate standardized digestibility) and diet specific losses (to estimate true digestibility; NRC, 2012). In the past, ELF have been estimated from regression models, by feeding decreasing dietary fat levels and extrapolating fecal fat to 0 dietary fat intake

(Jorgensen et al., 1993). This method estimates both basal and diet specific ELF for the determination of true total tract digestibility. Currently, there is a paucity of data on the basal losses of ELF and standardized total tract digestibility (**STTD**) of dietary fat.

Digestible energy (DE) corrects GE for the portion of energy that is contained in fecal matter (Patience, 2012). Currently, dietary fat DE content is determined on an apparent digestibility basis (NRC, 2012). Therefore, if a substantial portion of the fat contained in feces is of ELF origin, estimates of the DE content of dietary fat will be overcorrected the for energy losses contained in fecal matter; consequently, DE will be underestimated.

The objectives of this experiment were 1) to estimate basal ELF by feeding a fat-free diet, 2) to determine the apparent total tract digestibility (**ATTD**) and STTD of 14 dietary fat sources, and 3) to quantify the underestimation of the energy content of dietary fat when ELF is not considered.

Materials and methods

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research, and were approved by the Iowa State University Institutional Animal Care and Use Committee (#2-16-8201-S).

Animals, Housing, and Experimental Design

Determination of ATTD and STTD of acid hydrolyzed ether extract (**AEE**) was carried out using 120 Genetiporc 6.0 × Genetiporc F25 (PIC, Inc., Hendersonville, TN) barrows in 2 replicates of 60 barrows each. Barrows (d 0 BW of 9.9 ± 0.6 kg) were randomly allotted to 1 of 15 dietary treatments: control (**CNTR**), animal-vegetable blend (**AV**), canola oil (**CANO**),

choice white grease source A (**CWGA**), choice white grease source B (**CWGB**), coconut oil (**COCO**), corn oil source A (**CORA**), corn oil source B (**CORB**), fish oil (**FISH**), flaxseed oil (**FLAX**), palm oil (**PALM**), poultry fat (**POUF**), soybean oil source A (**SOYA**), soybean oil source B (**SOYB**), and tallow (**TAL**). An additional 8 barrows (average initial BW of 9.9 ± 0.6 kg) were utilized to determine ELF. Pigs were housed individually throughout the 56 d experiment.

Diets and Feeding

Each experimental diet contained 95% of a corn-soybean meal basal diet plus 5% of either: corn starch (CNTR) or 1 of the 14 dietary fat sources listed previously (Table 6.1). Dietary fat sources were selected to provide a diverse range of fatty acid saturation. The chemical composition and the fatty acid profile of the sources of dietary fat are described in Kellner et al. (2017). The fat-free (AEE ≤ 0.28%) diets used to estimate ELF are presented in Table 6.2.

Pigs were fed their assigned diets from d 0 to 10 and d 46 to 56. These experimental periods provided a 7 d acclimation to the diet prior to fecal collection. Pigs were fed the same fat source or dietary treatment in both experimental periods and fed a common diet between experimental periods (d 10 to 46; Kellner et al., 2017). Feed was limited from d 0 to 10 to provide an estimated daily energy intake of 2.8 times maintenance (NRC, 2012). From d 10 to 46, feed was provided ad libitum. Feed was limited from d 46 to 56 to provide a daily energy intake of 3.2 times maintenance (NRC, 2012). Feed allowances were selected for each phase to maximize intake without having variation of feed intake among pigs. Water was provided *ad* libitum at all times from d 0 to 56.

Representative feed samples were collected at mixing and stored at -20°C. Representative dietary fat samples were collected by subsampling from a minimum of 5 different locations. The subsamples of dietary fat were taken from the top, middle, and bottom, as well as, the center and peripheral of the container of fat. These samples were homogenized and stored at -20°C to provide a representative sample for later analysis. Prior to the initiation of the experiment, pigs were fed a common post-weaning nursery diet.

Data and Sample Collection

Pigs were individually weighed on d 0, 7, 10, 22, 46, 53, and 56. Fresh fecal grab samples were harvested from 0800 to 1000 h and 1600 to 1800 h on d 7 to 10 and d 53 to 56. Fecal samples were immediately stored at -20°C.

Analytical Methods

Feed and fecal samples were homogenized, dried, and then finely ground through a 1 mm screen in a Retsch grinder (model ZMI; Retsch Inc., Newtown, PA). All feed analyses were performed in duplicate unless otherwise noted and repeated when the sample average difference was greater than 1%. Acid hydrolyzed ether extract (method 2003.06; AOAC, 2007) was analyzed using a SoxCap SC 247 hydrolyzer and a Soxtec 255 semiautomatic extractor (FOSS North America, Eden Prairie, MN). Dry matter was determined by drying samples in an oven at 105°C to a constant weight. Gross energy was determined using an isoperibolic bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). Benzoic acid (6.318 Mcal/kg; Parr Instrument Co.) was used as the standard for calibration and determined to contain 6.319 ± 0.005

Mcal of GE/kg. Titanium dioxide was determined by spectrophotometer (synergy 4; BioTek Instruments Inc., Winooski, VT) according to the method of Leone (1973).

Calculations

The apparent total tract digestibility (ATTD; %) of AEE was calculated as 100 – {100 × [concentration (g) of TiO2 in diet × concentration of (g) of AEE in feces]/[concentration (g) of TiO2 in feces × concentration of (g) AEE in diet]} (Oresanya et al. 2007). The ELF was determined in pigs fed fat-free diets by the following equation: ELF (g/kg of DM intake) = [g of AEE/kg of feces] - [g of AEE/kg of feed]. The standardized total tract digestibility (STTD; %) of AEE was calculated as ATTD of AEE, % + {[ELF (g/kg of DM intake)/concentration (g) of AEE in diet] × 100} (Stein et al., 2007).

Determination of the energy content of dietary fat sources was based on the ATTD or STTD of AEE. The DE content of dietary fat not corrected for ELF was calculated by the following equation: apparent $DE_{dietary\ fat}$ (Mcal/kg) = $GE_{dietary\ fat}$ (9.4 Mcal/kg; Atwater and Bryant, 1900; NRC, 2012) × ATTD of AEE. The DE content of dietary fat corrected for ELF was calculated by the following equation: corrected $DE_{dietary\ fat}$ (Mcal/kg) = $GE_{dietary\ fat}$ (9.4 Mcal/kg; Atwater and Bryant, 1900; NRC, 2012) × STTD of AEE. Further determination of ME and NE content of dietary fat was calculated by $ME_{dietary\ fat}$ (Mcal/kg) = DE × 98% and $NE_{dietary\ fat}$ (Mcal/kg) = ME × 88% (van Milgen et al., 2001; NRC, 2012).

Statistical Analysis

Least square means and standard error for ELF of AEE digestion across the total tract were generated using PROC MIXED (SAS 9.4; SAS Inst. Inc., Cary, NC). The treatment effects

on ATTD and STTD of AEE and the energy content of dietary fat were analyzed using PROC MIXED with dietary treatment (n = 15) as a fixed effect, replicate (n = 2 × 60 barrows each) as a random effect, and pig (n = 120) as the experimental unit. For each variable, normal distribution of residuals was tested using PROC UNIVARIATE.

Non-detectable fatty acid concentrations were treated in all statistical analyses as 0. All *P*-values equal or less than 0.050 were considered significant and *P*-values between 0.050 and 0.100 were considered trends.

Results

Estimation of total tract ELF

Estimated ELF at 9 kg BW (d 7 to 10) was 4.17 g/kg of DM intake ($P < 0.001$; Table 6.3). Estimated ELF at 38 kg BW (d 53 to 56) was 6.67 g/kg of DM intake ($P = 0.002$). This represented a 46% increase in ELF from the lighter BW.

Effects of dietary fat source on ATTD and STTD of AEE

Adding 5% dietary fat regardless of source compared to pigs fed CNTR (5% corn starch) increased the ATTD of AEE and the STTD of AEE at both fecal collection time points ($P < 0.001$; Table 6.4). Among diets with fat added, the ATTD of AEE at 13 kg BW was the greatest in pigs fed CANO- and FISH-based diets and was the least in pigs fed PALM- and TAL-based diets ($P < 0.001$). The STTD of AEE was the greatest in barrows fed CANO-, CWGA-, and FISH-based diets and was the least in pigs fed PALM- and TAL-based diets ($P < 0.001$). The range of STTD of AEE among dietary fat sources analyzed at 13 kg BW was 3.20%. At 50 kg BW, ATTD and STTD of AEE was the greatest in pigs fed a CANO-based diet and the least in

pigs fed a CORA-based diet ($P < 0.001$; Table 6.4). The range of STTD of AEE among dietary fat sources analyzed at 50 kg BW was 4.22%.

Table 6.5 provides the g/kg of DM intake of AEE intake, disappearance, and fecal excretion used to determine the ATTD and STTD of AEE of the 15 dietary treatments. Furthermore, it shows ELF on average accounted for 43.1% and 68.0% of the fecal AEE at both collection time points, respectively.

Apparent and corrected energy content of dietary fat

Due to the method of calculation, dietary fat source had the same impact on the dietary fat energy content as it had on ATTD and STTD of AEE (Table 6.6). At 13 kg BW, the average apparent energy content of the 14 dietary fat sources was 8.39, 8.28, and 7.23 of DE, ME and NE of Mcal/kg, respectively. After the correction for ELF contained in fecal matter, the corrected energy content of the 14 dietary fat sources was 8.81, 8.63, and 7.60 of DE, ME and NE of Mcal/kg, respectively.

At 50 kg BW, the average apparent energy content of the 14 dietary fat sources was 8.50, 8.33, and 7.33 of DE, ME and NE of Mcal/kg, respectively (Table 6.6). After the correction for ELF, the corrected energy content of the 14 dietary fat sources was 9.10, 8.92, and 7.85 of DE, ME and NE of Mcal/kg, respectively. The difference between the apparent and corrected DE content of dietary fat was 0.42 and 0.60 Mcal/kg at 13 and 50 kg BW, respectively.

Discussion

ELF estimation

Endogenous losses can be estimated as basal losses - non-diet specific losses - to estimate standardized digestibility or diet specific losses - to estimate true digestibility (NRC, 2012).

Basal ELF, used to determine STTD of AEE, was measured in this experiment by feeding a fat free diet. This compares to the regression method, in which increasing dietary fat levels are fed (Jorgensen et al., 1993); regressing fat intake to 0 provides an estimate of diet specific ELF which is then used to calculate true total tract digestibly of AEE. In this experiment, the estimate for ELF over the total tract was 4.17 g/kg of DM intake for 9 kg BW barrows, and 6.67 g/kg of DM intake for 38 kg BW barrows. The BW of pigs used to estimate ELF were slightly less than the BW of pigs used to estimate ATTD and STTD of AEE. The explanation for this discrepancy is both groups of pigs started the 7 d adaption period (d 0 and d 46) at a similar BW, but the pigs fed the purified, fat-free diet grew much more slowly during the 10 d of feeding the experimental diets. This resulted in the observed BW difference during the fecal collection periods.

Estimates of ELF over the total tract via the regression method have been carried out using both endogenous fat - naturally present within the cereal grain - and exogenous fat - fat added to the diet as an energy supplement (Acosta et al., 2015). Previous diet specific ELF have been estimated at 4.4 (~6 kg BW; Adams and Jensen, 1984), 4.4 (~75 kg BW; Jorgensen et al., 1993), 22.4 (~63 kg BW; Jorgensen and Fernandez, 2000), 3.8 (~38 kg BW; Kil et al., 2010) and 6.5 g/kg of DM intake (~52 kg BW; Kim et al., 2013). A recent estimate of diet specific ELF in diets containing both intact and extracted fat was reported to be 13.6 g/kg of DM intake in 34 kg BW pigs (Gutierrez et al., 2016). The estimated basal losses of ELF reported herein at 4.17 and 6.67 g/kg of DM intake, at 9 and 38 kg BW, respectively, cannot be directly compared to the estimates listed above and reported in the literature.

Impact of ELF on the energy content of dietary fat

The implication of ELF accounting for ~40% to 70% of the AEE excreted in feces is that the current energy content estimates of dietary fat are underestimated. Digestible energy (DE) corrects GE for the portion of energy that is contained in fecal matter (Patience, 2012). The correction from GE to DE is done on an apparent digestibility basis (NRC, 2012). Thus, the correction from GE to DE does not distinguish the proportion of energy contained in feces between dietary or endogenous origin. These ELF and STTD of AEE data herein indicate on average that 43.1% and 68.0% (at 13 and 50 kg of BW, respectively) of the fat-based energy contained in feces is of endogenous origin and not dietary fat origin. Using a GE content of dietary fat of 9.4 Mcal/kg (Atwater and Bryant, 1900; NRC, 2012) and the ATTD and STTD of AEE observed herein, it was determined that by not correcting for ELF, the DE content of dietary fat is underestimated by 0.42 and 0.60 Mcal/kg at 13 and 50 kg of BW, respectively.

However, there are 2 issues associated with the application of corrected or true DE content of dietary fat. First, the DE content of almost all ingredients does not account for endogenous losses of digestion (NRC, 2012). Due to previous determinations of DE content being measured on an apparent digestibility basis (NRC, 2012). Thus, a correction of the DE content of just 1 ingredient or a single group of ingredients (i.e., dietary fat sources) would create difficulties in comparing the DE content among all the ingredients. This issue clearly exposes a weakness of the DE system. The correction from GE to DE incorrectly assumes that all energy contained in fecal matter is of dietary origin. The exposure of this weakness of the DE system raises 2 additional inquiries. First, is the energy contained in fecal matter of endogenous losses of fat or other nutrients equal across ingredients? If the answer to the previous question is no, then one could hypothesize that comparison across ingredients using the DE system is not

accurate. Second, is the ME content of ingredients is determined in most all instances by correcting the DE content for urinary energy losses (NRC, 2012). Thus, if the DE content of an ingredient is underestimated, then the ingredient ME content is also underestimated. More work is needed to validate if the DE content of dietary fat and other ingredients needs to be corrected for endogenous losses. The second issue with the application of corrected or true DE content of dietary fat arises in the large intestine of pigs. Unsaturated fatty acids are biohydrogenated by microbes (Jorgensen et al., 1993). This microbial process replaces double bonds between carbons in the fatty acid chain with carbon and hydrogen bonds (Jorgensen et al., 1993). This results in fat contained in fecal matter to be more saturated then when measured at the ileum or in feed (Just et al., 1980). Furthermore, the energy required to oxidize a saturated versus an unsaturated bond is different (Blanksby and Ellison, 2003). Therefore, the actual fecal ELF caloric content may be in error due to microbial modification.

Conclusion

In summary, the average STTD of AEE of these 14 dietary fat sources was 93.7% and 96.8% at 13 kg BW and 50 kg BW respectively. On average ELF accounted for 43.1% and 68.0% of the AEE contained in feces at 13 and 50 kg respectively. The substantial proportion of AEE contained in feces that is of ELF origin and not of dietary origin implies that the current estimates of the DE content of dietary fat are underestimated. Additionally, this implicated that the assumption that all energy contained in fecal matter is of dietary origin is a major flaw of the DE system. It was determined that by not correcting for ELF, the dietary fat DE content is underestimated by 0.42 and 0.60 Mcal/kg at 13 and 50 kg of BW, respectively.

Literature cited

Acosta, J. A., R. D. Boyd, and J. F. Patience. 2015. Endogenous and exogenous fat digestion in growing pigs. J. Anim. Sci. 93(Suppl 2):75.

Adams, K. L., and A. H. Jensen. 1984. Comparative utilization of in-seed fats and the respective extracted fats by the young pig. J. Anim. Sci. 59:1557-1566. doi:10.2527/jas1984.5961557x.

AOAC Int. 2007. Official methods of analysis. 18th rev. ed. AOAC Int, Gaithersburg, MD.

Atwater, W. O., and A. P. Bryant. 1900. Availability and fuel value of food materials. In: 1899 annual report of Storrs Agr. Exp. Sta. p 70-100.

Blanksby, S. J, and G. B. Ellison. 2003. Bond dissociation energies of organic molecules. Acc. Chem. Res. 36:255-263.

Gutierrez, N. A., N. V. L. Serao., and J. F. Patience. 2016. Effects of distillers' dried grains with solubles and soybean oil on dietary lipid, fiber, and amino acid digestibility in corn-based diets fed to growing pigs. J. Anim. Sci. 94:1508-1519. doi:10.2527/jas.2015-9529.

Jorgensen, H., and J. A. Fernandez. 2000. Chemical composition and energy value of different fat sources for growing pigs. Acta Agric. Scand. A Anim. Sci. 50:129-136.

Jorgensen, H., K. Jakobsen, and B. O. Eggum. 1993. Determination of endogenous fat and fatty acids at the terminal ileum and on faeces in growing pigs. Acta Agric. Scand. A Anim. Sci. 43:101-106.

Just, A., J. O. Andersen, and H. Jørgensen. 1980. The influence of diet composition on the apparent digestibility of crude fat and fatty acids at the terminal ileum and overall in pigs. Z. Tierphyphysiol. Tierernaehr. Futtermittelkde. 44:82–92.

Kellner, T. A., and J. F. Patience. 2017. The definition and the explanation of the DE, ME and NE content of dietary fat sources in 13 and 50 kg pig. J. Anim. Sci. (in-preparation).

Kil, D. Y., T. E. Sauber, D. B. Jones, and H. H. Stein. 2010. Effect of the form of dietary fat and the concentration of dietary neutral detergent fiber of ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. J. Anim. Sci. 88:2959-2967. doi:10.2527/jas.2009-2216.

Kim, B. G., D. Y. Kil, and H. H. Stein. 2013. In growing pigs, the true and total tract digestibility of acid hydrolyzed ether extract in extracted corn oil is greater than in intact sources of corn oil or soybean oil. J. Anim. Sci. 91:755-763. doi:10.2527/jas.2011-4777.

Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. J. Assoc. Off. Anal. Chem. 56:535–537.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Oresanya, T. F., A. D. Beaulieu, E. Beltranena, and J. F. Patience. 2007. The effect of dietary energy concentration and total lysine/digestible energy ratio on the growth performance of weaned pigs. Can. J. Anim. Sci. 87:45–55. doi:10.4141/A05-064.

Patience, J. F. 2012. The influence of dietary energy on feed efficiency in grow-finish swine. In: J. F. Patience, editor, Feed efficiency in swine. Wageningen Academic Publishers, Wageningen, the Netherlands. p. 101-129.

van Milgen, J., J. Noblet, and S. Dubois. 2001. Energetic efficiency of starch, protein, and lipid utilization in growing pigs. J. Nutr. 131:1309-1318.

Table 6.1. Ingredient and nutrient composition (as-fed basis) of the experimental diets from d 0 to 10 and d 46 to 56

	d 0 to 10		d 46 to 56	
Item	Control	5% Dietary fat	Control	5% Dietary fat
Ingredient, %				
Corn	59.90	59.90	68.41	68.41
Soybean meal (46.5% CP)	20.00	20.00	22.50	22.50
Corn Starch	5.00	-	5.00	-
Experimental dietary fat	-	5.00	-	5.00
Whey, permeate	6.20	6.20	-	-
Plasma (spray-dried)	5.00	5.00	-	-
Limestone	1.09	1.09	0.96	0.96
Monocalcium phosphate (21%)	0.84	0.84	1.22	1.22
Salt	0.25	0.25	0.50	0.50
L-lysine HCL	0.43	0.43	0.33	0.33
DL-methionine	0.18	0.18	0.10	0.10
L-threonine	0.14	0.14	0.12	0.12
L-isoleucine	0.06	0.06	-	-
L-valine	0.04	0.04	-	-
Trace mineral premix[2]	0.20	0.20	0.20	0.20
Vitamin premix[3]	0.20	0.20	0.20	0.20
Santoquin[4]	0.06	0.06	0.06	0.06
Titanium dioxide	0.40	0.40	0.40	0.40
Analyzed composition				
DM, %	88.12	88.79	88.76	89.00
Acid hydrolyzed ether extract, %	2.63	8.26	7.69	8.20

[1]Experimental dietary fats were: animal-vegetable blend (sourced via Darling Pro Ingredients [Wahoo, NE]), canola oil (sourced via Bulk Apothecary [Aurora, OH]), choice white grease source A (sourced via JBS [Marshalltown, IA]), choice white grease source B (sourced via JBS [Worthington, MN]), coconut oil (sourced via Bulk Apothecary), corn oil source A (sourced via Feed Energy Co. [Des Moines, IA]), corn oil source B (sourced via Double S Liquid Feed Services [Danville, IL]), fish oil (sourced via Double S Liquid Feed Services), flaxseed oil (sourced via Double S Liquid Feed Services), palm oil (sourced via Bulk Apothecary), poultry fat (sourced via Boyer Valley Co. [Denison, IA]), soybean oil source A (sourced via Status Foods [Memphis, TN]), soybean oil source B (sourced via Bulk Apothecary), tallow (sourced via Darling Pro Ingredients [Omaha, NE]).

[2]Provided 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kilogram of diet.

[3]Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.023 mg vitamin B12 per kilogram of diet.

[4]Santoquin Mixture 6 (feed and forage antioxidant; Novus International, St. Charles, MO).

Table 6.2. Ingredient and nutrient composition (as-fed basis) of fat-free experimental diets d 0 to 10 and d 46 to 56

Item	d 0 to 10	d 46 to 56
Ingredient, %		
Corn starch	76.96	78.53
Sucrose	10.00	10.00
Solka Floc	3.00	3.00
L-lysine HCL	1.65	1.25
DL-methionine	0.75	0.57
L-threonine	0.80	0.62
L-tryptophan	0.22	0.17
L-isoleucine	0.72	0.54
L-valine	0.86	0.66
Monocalcium phosphate (21%)	1.90	1.80
Limestone	0.97	0.92
Salt	0.68	0.50
Potassium carbonate	0.50	0.45
Magnesium oxide	0.02	-
Trace mineral premix[2]	0.20	0.20
Vitamin premix[3]	0.31	0.31
Santoquin[4]	0.06	0.06
Titanium dioxide	0.40	0.40
Analyzed composition		
DM, %	92.01	91.53
Acid hydrolyzed ether extract, %	0.18	0.28

[1]Feed to all pigs from d 10 to 46 regardless of treatment assigned.

[2]Provided 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kilogram of diet.

[3]Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.023 mg vitamin B12 per kilogram of diet.

[4]Santoquin Mixture 6 (feed and forage antioxidant; Novus International, St. Charles, MO).

Table 6.3. Least square means of the estimated endogenous losses of dietary fat digestion (ELF) across the total tract

Item	Estimated ELF, g/kg of DM intake[1]	*P*-value
9 kg BW[2]	4.17 ± 0.69	<0.001
38 kg BW[3]	6.67 ± 1.11	0.002

[1]Estimate of ELF was determined by the following equation: ELF = [g of acid hydrolyzed ether extract/kg of feces (dry matter basis)] - [g of acid hydrolyzed ether extract/kg of feed (dry matter basis)].

[2]Determined via 8 pigs with a d 7 BW of 9.1 ± 0.6 kg and a d 10 BW of 9.2 ± 0.6 kg.

[3]Determined via 8 pigs with a d 53 BW of 37.6 ± 2.1 kg and a d 56 BW of 37.6 ± 2.2 kg.

Table 6.4. Effects of dietary fat source on apparent total tract digestibility (ATTD) of acid hydrolysis ether extract (AEE)[1], and standardized total tract digestibility (STTD)[2] of dietary AEE at 13 and 50 kg BW

	Dietary treatment[3]															
Item	CNTR	AV	CANO	CWGA	CWGB	COCO	CORA	CORB	FISH	FLAX	PALM	POUF	SOYA	SOYB	TAL	SEM	*P*-value
13 kg BW[3] ATTD of AEE, %	74.0^{d}	89.0^{abc}	90.8^{a}	90.2^{ab}	90.0^{ab}	89.6^{abc}	88.1^{bc}	88.3^{bc}	91.0^{a}	88.2^{bc}	87.7^{c}	89.7^{abc}	89.4^{abc}	89.3^{abc}	87.7^{c}	0.8	<0.001
STTD of AEE,[4] %	88.0^{e}	93.3^{abcd}	95.1^{ab}	95.0^{ab}	94.5^{abc}	94.2^{abcd}	92.9^{bcd}	93.0^{bcd}	95.4^{a}	92.7^{cd}	92.2^{d}	94.1^{abcd}	94.0^{abcd}	93.6^{abcd}	92.1^{d}	0.8	<0.001
50 kg BW[5] ATTD of AEE, %	73.5^{e}	89.5^{c}	92.5^{a}	90.8^{abc}	91.7^{ab}	90.5^{bc}	87.6^{d}	90.0^{bc}	91.1^{abc}	91.5^{ab}	89.4^{c}	90.4^{bc}	91.7^{ab}	90.4^{bc}	89.4^{c}	1.0	<0.001
STTD of AEE,[6] %	93.0^{g}	95.8^{def}	98.6^{a}	97.1^{abcde}	98.2^{ab}	97.0^{abcde}	94.3^{f}	96.6^{bcde}	97.5^{abcd}	97.7^{abc}	95.9^{cdef}	96.8^{abcde}	97.7^{abc}	96.5^{bcde}	95.7^{ef}	1.0	<0.001

[1]Apparent total tract digestibility (ATTD; %) of AEE was calculated as 100 – {100 × [concentration (g) of TiO_2 in diet × concentration of (g) of AEE in feces]/[concentration (g) of TiO_2 in feces × concentration of AEE in diet]}; (Oresanya et al. 2007).

[2]Calculated as ATTD of AEE (%) + {[ELF (g/kg of DM intake)/concentration (g) of AEE in diet] × 100} (Stein et al., 2007).

[3]Each experimental diet included 95% of a corn-soybean meal basal diet and then 5% of either: corn starch (CNTR), animal-vegetable blend (AV), canola oil (CANO), choice white grease source A (CWGA), choice white grease source B (CWGB), coconut oil (COCO), corn oil source A (CORA), corn oil source B (CORB), fish oil (FISH), flaxseed oil (FLAX), palm oil (PALM), poultry fat (POUF), soybean oil source A (SOYA), soybean oil source B (SOYB), or tallow (TAL).

[4]Determined via 120 pigs (8 pigs/treatment) with a d 7 BW of 12.3 ± 0.2 kg and a d 10 BW of 13.8 ± 0.4 kg.

[5]Calculated by correcting ATTD of AEE (%) for endogenous losses estimated at 4.17 g/kg of DM intake.

[6]Determined via 120 pigs (8 pigs/treatment) with a d 53 BW of 49.1 ± 2.2 kg and a d 56 BW of 51.7 ± 1.7 kg.

[7]Calculated by correcting ATTD of AEE (%) for endogenous losses estimated at 6.67 g/kg of DM intake.

Table 6.5. Effects of dietary fat source on intake, disappearance, and fecal excretion of acid hydrolyzed ether extract (AEE; g/kg of DM intake) from d 7 to 10 and d 53 to 56

	Dietary treatment[3]																
Item	CNTR	AV	CANO	CWGA	CWGB	COCO	CORA	CORB	FISH	FLAX	PALM	POUF	SOYA	SOYB	TAL	SEM	*P*-value
d 7 to 10																	
Intake	29.8	99.1	97.6	86.6	92.1	90.2	87.3	89.9	95.1	93.5	93.1	92.4	91.8	97.9	95.6	-	-
TTD[1]	22.1^{i}	88.2^{ab}	88.6^{a}	78.1^{gh}	82.9^{cd}	80.8^{ef}	76.9^{h}	79.4^{fg}	86.6^{b}	82.4^{cde}	81.7^{de}	82.8^{cde}	82.1^{cde}	87.4^{ab}	83.9^{c}	0.7	<0.001
TFE[2]	7.7^{f}	10.9^{abcd}	9.0^{def}	8.5^{ef}	9.2^{cdef}	9.4^{cdef}	10.4^{abcde}	10.5^{abcde}	8.5^{ef}	11.0^{abc}	11.5^{ab}	9.6^{bcdef}	9.7^{bcdef}	10.5^{abcde}	11.7^{a}	0.7	0.002
ELF[3] in feces	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	-	-
Non-ELF in feces[4]	3.6^{f}	6.7^{abcd}	4.8^{def}	4.3^{ef}	5.1^{cdef}	5.2^{cdef}	6.2^{abcde}	6.4^{abcde}	4.4^{ef}	6.8^{abc}	7.3^{de}	5.4^{cde}	5.5^{cde}	6.3^{ab}	7.6^{a}	0.7	0.002
d 7 to 10																	
Intake	34.2	106.7	109.4	106.3	103.5	101.9	98.6	102.0	104.3	108.3	103.3	104.5	110.1	109.5	105.4	-	-
TTD[1]	25.2^{g}	95.6^{cd}	101.1^{a}	96.5^{d}	94.9^{d}	92.1^{e}	86.3^{f}	91.9^{e}	94.9^{d}	99.1^{b}	92.3^{e}	94.5^{d}	101.0^{a}	99.0^{b}	94.2^{d}	1.0	<0.001
TFE[2]	9.1^{cde}	11.2^{ab}	8.2^{e}	9.8^{bcde}	8.6^{d}	9.7^{bcde}	12.3^{a}	10.2^{bc}	9.3^{cde}	9.2^{cde}	10.9^{ab}	10.1^{bcd}	9.2^{cde}	10.5^{bc}	11.2^{ab}	1.0	<0.001
ELF[3] in feces	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	-	-
Non-ELF in feces4	2.4^{cde}	4.5^{ab}	1.6^{e}	3.1^{bcde}	1.9^{d}	3.1^{bcde}	5.6^{a}	3.5^{bc}	2.7^{cde}	2.5^{cde}	4.3^{ab}	3.4^{bcd}	2.5^{cde}	3.8^{bc}	4.5^{ab}	1.0	<0.001

[1]Total tract disappearance (g/kg of DM intake) = intake – total amount of AEE in feces.

[2]Total fecal excretion

[2]Basal endogenous losses of fat digestion (g/kg of DM intake).

[3]Amount of AEE from non-ELF origin (g/kg of DM intake) was calculated as total amount of AEE in feces – ELF in feces.

Table 6.6. Determination of DE, ME and NE content of dietary fat sources (Mcal/kg; as-fed basis) based on the apparent total tract digestion (ATTD) or standardized total tract digestion (STTD) of acid hydrolyzed ether extract (AEE) at 13 and 50 kg BW[1]

	Dietary treatment[2]															
Item	AV	CANO	CWGA	CWGB	COCO	CORA	CORB	FISH	FLAX	PALM	POUF	SOYA	SOYB	TAL	SEM	*P*-value
13 kg BW																
Apparent energy content																
DE[3]	8.37^{abc}	8.53^{a}	8.48^{ab}	8.46^{ab}	8.42^{abc}	8.28^{bc}	8.30^{bc}	8.56^{a}	8.29^{bc}	8.24^{c}	8.42^{abc}	8.41^{abc}	8.40^{abc}	8.25^{c}	0.08	0.041
ME[4]	8.20^{abc}	8.36^{a}	8.31^{ab}	8.29^{ab}	8.28^{abc}	8.12^{bc}	8.14^{bc}	8.38^{a}	8.13^{bc}	8.08^{c}	8.26^{abc}	8.24^{abc}	8.23^{abc}	8.08^{c}	0.07	0.041
NE[5]	7.22^{abc}	7.36^{a}	7.31^{ab}	7.29^{ab}	7.26^{abc}	7.14^{bc}	7.16^{bc}	7.38^{a}	7.15^{bc}	7.11^{c}	7.26^{abc}	7.25^{abc}	7.24^{abc}	7.11^{c}	0.06	0.041
Corrected energy content																
DE[6]	8.76^{abcd}	8.94^{ab}	8.93^{ab}	8.88^{abc}	8.85^{abcd}	8.73^{bcd}	8.74^{bcd}	8.97^{a}	8.72^{cd}	8.66^{d}	8.85^{abcd}	8.83^{abcd}	8.80^{abcd}	8.66^{d}	0.08	0.045
ME[4]	8.59^{abcd}	8.76^{ab}	8.75^{ab}	8.70^{abc}	8.68^{abcd}	8.56^{bcd}	8.56^{bcd}	8.79^{a}	8.54^{cd}	8.49^{d}	8.67^{abcd}	8.66^{abcd}	8.62^{abcd}	8.48^{d}	0.07	0.045
NE[5]	7.56^{abcd}	7.71^{ab}	7.70^{ab}	7.66^{abc}	7.64^{abcd}	7.53^{bcd}	7.54^{bcd}	7.73^{a}	7.51^{cd}	7.47^{d}	7.63^{abcd}	7.62^{abcd}	7.59^{abcd}	7.47^{d}	0.06	0.045
50 kg BW																
Apparent energy content																
DE[3]	8.42^{c}	8.69^{a}	8.54^{abc}	8.62^{ab}	8.50^{bc}	8.23^{d}	8.46^{bc}	8.56^{abc}	8.60^{ab}	8.40^{c}	8.50^{bc}	8.62^{ab}	8.50^{bc}	8.40^{c}	0.09	<0.001
ME[4]	8.25^{c}	8.52^{a}	8.37^{abc}	8.45^{ab}	8.33^{bc}	8.07^{d}	8.29^{bc}	8.39^{abc}	8.43^{ab}	8.24^{c}	8.33^{bc}	8.45^{ab}	8.33^{bc}	8.23^{c}	0.09	<0.001
NE[5]	7.26^{c}	7.50^{a}	7.36^{abc}	7.43^{ab}	7.33^{bc}	7.10^{d}	7.30^{bc}	7.38^{abc}	7.42^{ab}	7.25^{c}	7.33^{bc}	7.43^{ab}	7.33^{bc}	7.25^{c}	0.08	<0.001
Corrected energy content																
DE[6]	9.00^{def}	9.26^{a}	9.13^{abcde}	9.23^{ab}	9.12^{abcde}	8.87^{f}	9.08^{bcde}	9.16^{abcd}	9.18^{abc}	9.01^{cdef}	9.10^{abcde}	9.19^{abc}	9.07^{bcde}	9.00^{ef}	0.09	<0.001
ME[4]	8.82^{def}	9.08^{a}	8.94^{abcde}	9.04^{ab}	8.94^{abcde}	8.69^{f}	8.90^{bcde}	8.98^{abcd}	9.00^{abc}	8.83^{cdef}	8.91^{abcde}	9.00^{abc}	8.89^{bcde}	8.82^{ef}	0.09	<0.001
NE[5]	7.77^{def}	7.99^{a}	7.87^{abcde}	7.96^{ab}	7.86^{abcde}	7.65^{f}	7.83^{bcde}	7.90^{abcd}	7.92^{abc}	7.77^{cdef}	7.84^{abcde}	7.92^{abc}	7.82^{bcde}	7.76^{ef}	0.08	<0.001

[1]Determined via 120 pigs (8 pigs/treatment) with a d 7 BW of 12.3 ± 0.2 kg and a d 10 BW of 13.8 ± 0.4 kg and a d 53 BW of 49.1 ± 2.2 kg and a d 56 BW of 51.7 ± 1.7 kg.

[2]Each experimental diet included 95% of a corn-soybean meal basal diet and then 5% of either: corn starch (CNTR), animal-vegetable blend (AV), canola oil (CANO), choice white grease source A (CWGA), choice white grease source B (CWGB), coconut oil (COCO), corn oil source A (CORA), corn oil source B (CORB), fish oil (FISH), flaxseed oil (FLAX), palm oil (PALM), poultry fat (POUF), soybean oil source A (SOYA), soybean oil source B (SOYB), or tallow (TAL).

[3]Apparent $DE_{dietary\ fat}$ (Mcal/kg) = $GE_{dietary\ fat}$ (9.4 Mcal/kg; Atwater and Bryant, 1900; NRC, 2012) × ATTD of AEE (refer to percentages in Table 6.4).

[4]$ME_{dietary\ fat}$ (Mcal/kg) = DE × 98% (van Milgen et al., 2001; NRC, 2012).

[5]$NE_{dietary\ fat}$ (Mcal/kg) = ME × 88% (van Milgen et al., 2001; NRC, 2012).

[6]Corrected $DE_{dietary\ fat}$ (Mcal/kg) = $GE_{dietary\ fat}$ (9.4 Mcal/kg; Atwater and Bryant, 1900; NRC, 2012) × STTD of AEE (refer to percentages in Table 6.4).

CHAPTER VII

INTEGRATIVE SUMMARY

General Discussion

The objective of adding fat to swine diets is to improve net income by increasing daily energy intake, thus enhancing feed efficiency and in many instances, growth rate. However, the pig's response to dietary fat is often assumed to be the same across sources, despite known chemical composition diversity. Furthermore, addition of fat to growing and finishing diets is often assumed to provide the same positive impact on growth and feed efficiency across differing environmental conditions and energy intakes. These assumptions have led to disappointment in predicting growth performance and carcass composition, ultimately resulting in overestimated financial returns for pork producers. Therefore, the overall objective of this dissertation was to discern which components of the chemical composition of dietary fat affects the digestibility and energy content of dietary fat; the expression of genes involved in lipid metabolism; and the resulting carcass fat composition. The specific sub-objectives were to validate that dietary linoleic acid concentration would be a superior predictor of carcass iodine value than iodine value product; determine if heat stress alters the pig's response to dietary fat; investigate the effect of chemical composition of dietary fat sources on transcriptional profiling of genes involved in lipid metabolism; develop regression equations that explain variation in the energy content of dietary fat sources based on chemical composition; quantify the underestimation of the energy content of dietary fat due to not accounting for endogenous losses of fat.

Apparent digestion of dietary fat can lead to incorrect interpretations across differing inclusion levels and intakes of dietary fat. For example, in Chapter 2 the apparent digestibility of

acid hydrolyzed ether extract (used instead of the more conventional ether extract to provide a more complete extraction; Palmquist and Jenkins, 2003) was increased as inclusion of dietary fat was increased from 2% to 6%. However, the increase in apparent digestibility was not due to dietary fat becoming more digestible with increased dietary concentration. The actual explanation for the increase in apparent digestibility was the dilution of endogenous losses of fat present in feces. Endogenous losses must be accounted for when comparing different inclusion levels of dietary fat. Reporting standardized or true digestibility of acid hydrolyzed ether extract allows for better comparison of results across experiments that included different levels of dietary fat.

It was found in both individually-fed pigs and group housed pigs that an unsaturated fat source (corn oil) was better digested than saturated fat sources (choice white grease and tallow in Chapter 2 and 3, respectively). Thus, the experiment in Chapter 5 and 6 was designed to discern which components of the chemical composition of dietary fats impact the digestibility of energy. Using 14 different dietary fat sources, it was determined that chemical composition of dietary fat explained ~80% of the variation observed in the energy content of dietary fat sources. The average DE content of the 14 dietary fat sources was 8.42 and 8.45 Mcal/kg at 13 kg BW and 50 kg BW, respectively. Powles et al. (1995) predicted that the average DE content of these sources would be 8.43 Mcal/kg. The Powles et al. (1995) equation has been considered the gold standard of prediction equations of dietary fat DE content and is currently used by the NRC (2012) in determining the DE, ME, and NE content of dietary fat sources. However, the observed DE values of dietary fat in Chapter 5 identified 2 potential weaknesses of the equation. The Powles et al. (1995) equation incorrectly predicted the DE content of saturated sources of dietary fat that are composed of fatty acid chain lengths < 16 carbons and underestimated the negative impact of

FFA. Step-wise linear regression was utilized to explain the relationship between the chemical composition of dietary fat and the observed DE values at 13 and 50 kg BW. It was unfair to compare these equations to the Powles et al. (1995) equation within this experiment as the regression equations were fitted to the same observed DE values from which they were generated. Thus, future research (preferably under commercial conditions) is needed to compare the equations generated in Chapter 5 to those reported by Powles et al. (1995) utilizing fat sources that are independent of both studies.

The DE system does not distinguish between fecal energy that is derived from the diet versus that of endogenous. In Chapter 6, it was found that endogenous losses of fat accounted for 43% and 68% of the acid hydrolyzed ether extract contained in feces at 13 and 50 kg BW, respectively. Thus, the digestible energy content of fat sources is underestimated when expressed on an apparent basis.

The chemical composition of dietary fat also has post-absorptive effects. It has been known for half a century that increased intake of dietary fat will suppress fatty acid synthase and the rate of de novo lipogenesis in growing pigs. It is less understood how the fatty acid composition of recently absorbed fat impacts lipogenesis and lipolysis. In Chapters 3 and 4, it was found that corn oil (a source with a high concentration of omega-6 fatty acids) was a less potent inhibitor of fatty acid synthase than other sources of dietary fat. In Chapter 4, it was found that increased intake of omega-3 fatty acids decreased the expression of genes involved in lipolysis in both adipose tissue and liver. In addition, due to their metabolic endpoint in the liver versus peripheral tissues, it was found that medium chain fatty acids have different effects than longer chain fatty acids on hepatic transcription of lipid metabolism genes. These data provide support for the mode of action explaining changes in carcass fat composition that were observed

in Chapters 2 and 3. In addition, these results bring into question the current assumption that the efficiency of converting DE to NE is same of all dietary fat sources.

It was first demonstrated nearly a century ago that the composition of dietary fat is highly reflected in the composition of pork carcass fat (Ellis and Isbell, 1926). It is therefore logical that the composition of pork carcass fat could be predicted from the composition of dietary fat. The first attempt at such a prediction was reported over 50 yr ago, resulting in the term "iodine value product," which is derived from an equation that includes both the iodine value of the dietary fat and the level of fat in the diet (Christensen, 1962; Madsen et al., 1992). Iodine value product is widely used in the pig industry as a tool to predict carcass iodine value. The weakness of the equation arises from the fact that both diet iodine value and the inclusion level of dietary fat are weighted equally. Depending on which dietary fat source is employed and how saturated or unsaturated it is, a 2% dietary fat level increase may have little to no impact or a very large impact on carcass iodine value (Kellner et al., 2014). As proposed in Benz et al. (2011) and Kellner et al. (2014), dietary linoleic acid concentration was validated in Chapter 2 to be a more precise predictor of carcass iodine value than iodine value product.

In summary, the chemical composition of dietary fat can be used to explain the variation in observed energy content and predict the resulting composition of pork carcass fat. The research in this dissertation validates that dietary linoleic acid concentration can be used to predict pork carcass iodine value. These data confirmed that adding dietary fat suppresses the expression of genes involved in lipogenesis and increases the expression of genes involved in lipolysis. This dissertation further detailed that increased intake of saturated fatty acids will suppress fatty acid synthase more than an increased intake of omega-6 fatty acids. It also reports that an increased intake of omega-3 fatty acids will decrease the abundance of protein kinase A

in adipose tissue and increased intake of medium chain fatty acids will impact hepatic lipogenesis and lipolysis differently than long chain fatty acids. Similar to what is found in the literature, digestibility of fat sources generally decreased as the free fatty acid level increased and unsaturated to saturated fatty acid ratio decreased. However, like past experiments the exact components of the chemical composition of dietary fat that impact the digestibility and energy content of dietary fat sources were inconsistent.

Recommendations for Future Research

It was determined that the chemical composition of dietary fat alters the mRNA abundance of key enzymes involved in both lipogenesis and lipolysis. This would indicate that the current assumption that the DE:NE ratio is equal across all dietary fat sources is incorrect. Furthermore, determination of NE of dietary fat via indirect calorimetry has only been done on 1 source (vegetable oil) at 1 level (7%) in 5 pigs (van Milgen et al., 2001). Clearly, more work is needed to validate the NE estimate of dietary fat and to determine the impact of the chemical composition of dietary fat on the metabolic heat produced by the growing pig.

The regression equations generated to explain the relationship between the chemical composition of dietary fat and observed DE values in Chapter 5 need to be validated (preferably under commercial conditions) and compared against the Powles et al. (1995) equation. The comparison of the equations should be done in experiments containing sources of dietary fat that provide a robust range of fatty acid compositions and free fatty acid content to expose any potential weaknesses. It was also determined that approximately half of the fat contained in feces was of non-dietary origin. More work is therefore needed to determine if the DE content of dietary fat needs to be adjusted for endogenous losses.

Finally, future research should be aimed at building a model that uses the chemical composition of dietary fat source and the energy intake of the pig, to explain observed lipid deposition rates (both from direct dietary fat deposition and de novo synthesized fatty acid origin) and carcass fatty acid compositions.

Literature cited

Benz, J. M., M. D. Tokach, S. S. Dritz, J. L. Nelssen, J. M. DeRouchey, R. C. Sulabo, and R. D. Goodband. 2011. Effects of dietary iodine value product on growth performance and carcass fat quality of finishing pigs. J. Anim. Sci. 89:1419–1428. doi:10.2527/jas.2010-3126.

Christensen, K. D. 1962. Foderfedtets indflydelse pa smorrets og flaeskets kvalitet. Thesis, Kgl. Vet.- og Landbohojsk., Kobenhavn, p. 88 (Danish).

Ellis, N. R., and H. S. Isbell. 1926. Soft Pork Studies. II. The influence of the character of the ration upon the composition of the body fat of hogs. J. Biol. Chem. 59:219-248.

Kellner, T. A., K. J. Prusa, and J. F. Patience. 2014. Impact of dietary fat source and concentration and daily fatty acid intake on the composition of carcass fat and iodine value sampled in three regions of the pork carcass. J. Anim. Sci. 92:5485-5495 doi:10.2527/jas.2014-7567.

Madsen, A., K. Jakobsen, and H. Mortensen. 1992. Influence of dietary fat on carcass fat quality in pigs. A review. Acta. Agric. Scand. 42:220-225.

NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Palmquist, D. L., and Jenkins T. C. 2003. Challenges with fats and fatty acid methods. J. Anim. Sci. 81:3250-3254. doi:10.2527/2003.81123250x.

Powles, J., J. Wiseman, D. J. A. Cole, and S. Jagger. 1995. Prediction of the apparent digestible energy value of fats given to pigs. Anim. Sci. 61:149-154. doi:1357-7298/95/45810149S20.00111.

van Milgen, J., J. Noblet, and S. Dubois. 2001. Energetic efficiency of starch, protein, and lipid utilization in growing pigs. J. Nutr. 131:1309-1318.